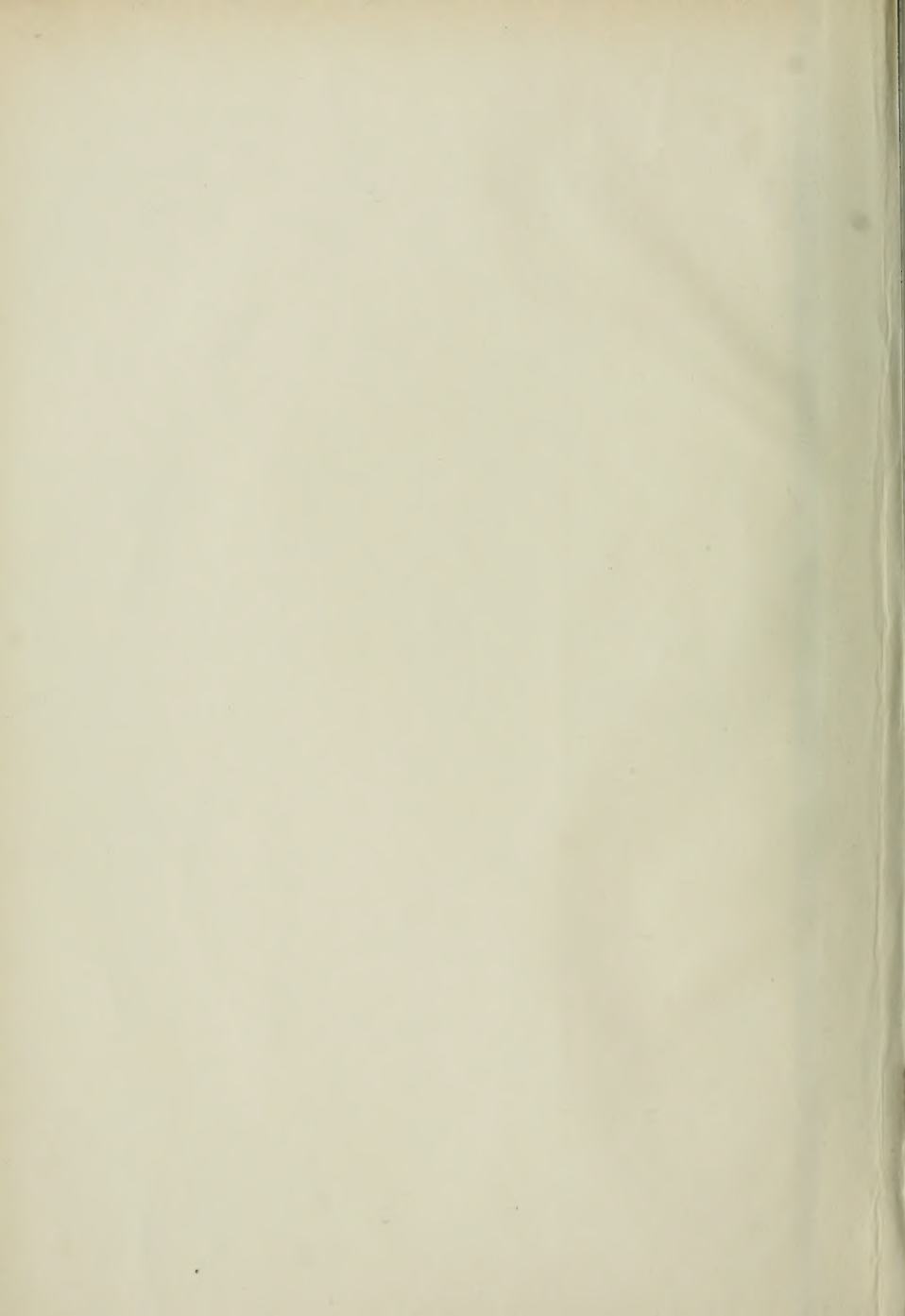


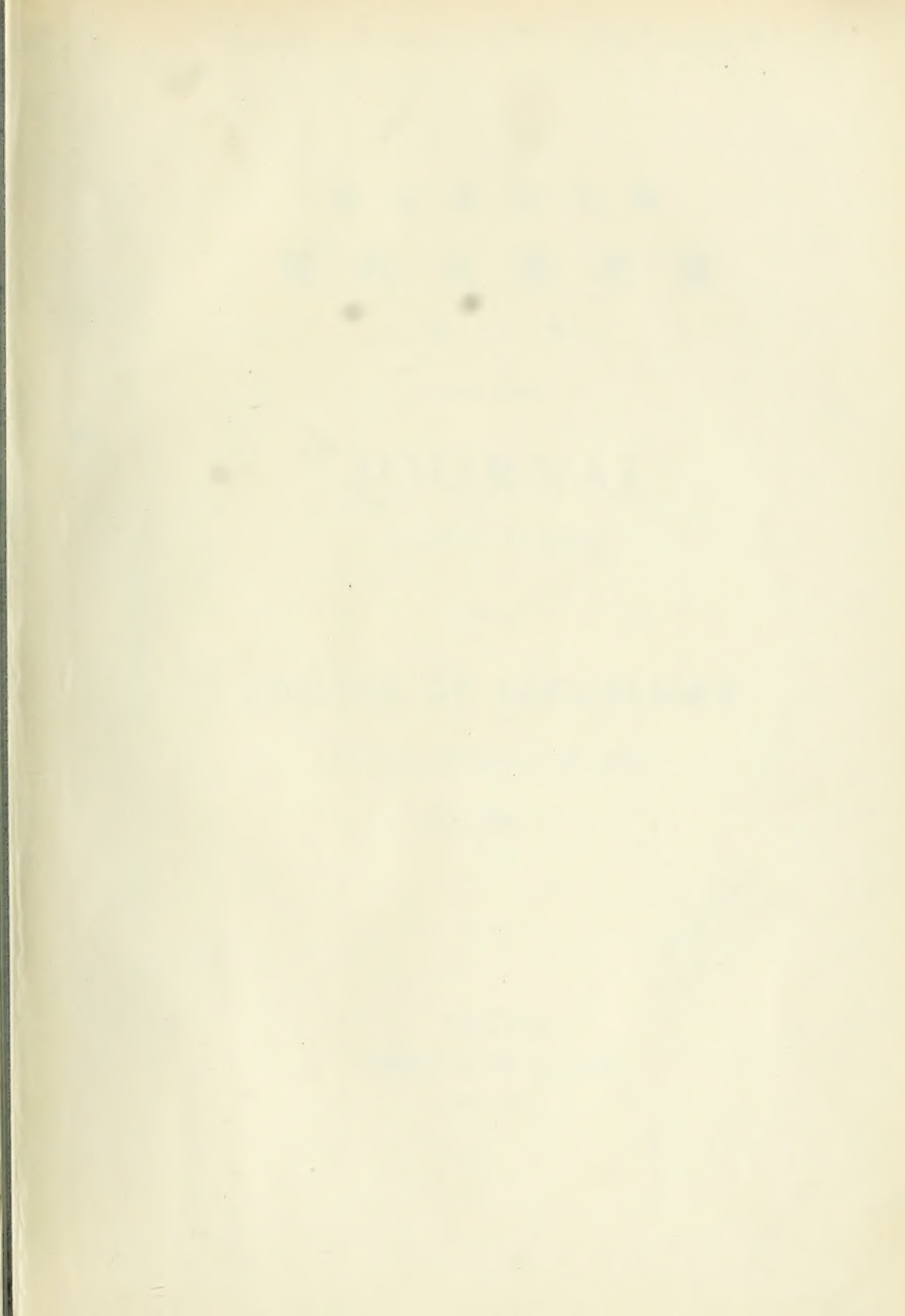
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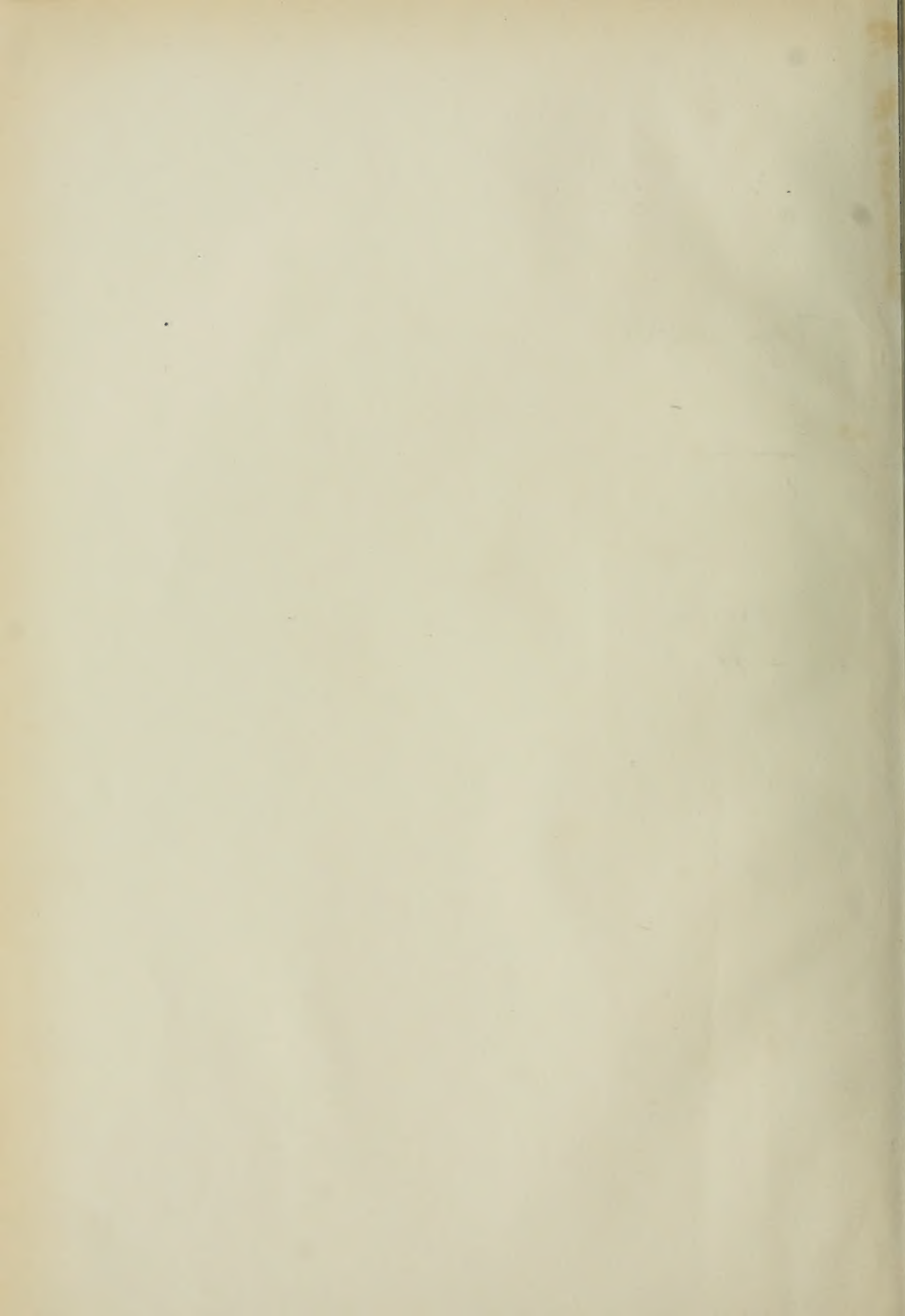












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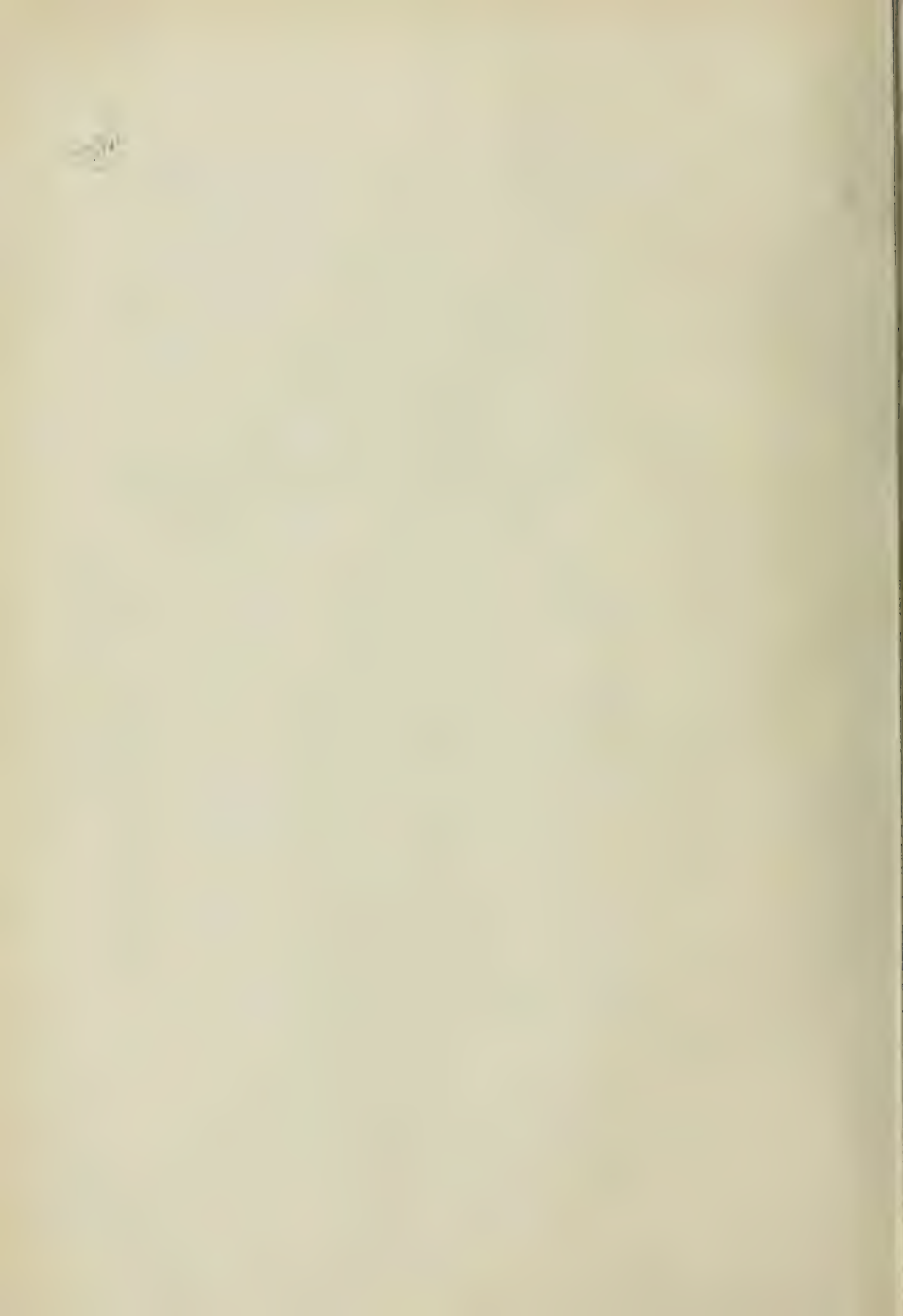
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## On the Occurrence of Urease in Higher Plants.

BY

T. Takeuchi.

---

Although much has been written about urease no mention is made of its occurrence in higher plants. Its presence has been proved only in Urobacteria and certain fungi. In the course of my investigations on a desamidizing enzyme in higher plants, I have discovered a very powerful urease in both the resting seeds and seedlings of soy-bean (*Glycine hispida*). The following observations on this point may, therefore, be not be without some value.

Experiment I. 30 seedlings of soy-bean about 3 cm. high, with their cotyledons removed were washed, crushed in a porcelain mortar, macerated with 50 c.c. water and filtered. Four such filtrates were prepared, each of them being put in an Erlenmeyer's flask of ca. 200 c.c. capacity. They were made faintly alkaline with 1 c.c. of normal soda-solution and covered with enough toluol. The special additions consisted in:

- (A) 0,5 g. Asparagine,
- (B) 0,5 g. Urea,
- (C) 0,5 g. Asparagine after boiling,
- (D) Control.

After 15 hours standing at 23°C in a thermostat they were taken out and each 5 c.c. of the filtrates was tested for ammonia with Nesler's reagent. The observations were as follows:

- (A) Very weak reaction,
- (B) Very strong reaction with brown precipitates,
- (C) No reaction,
- (D) No reaction.

The tests were repeated with a few modified additions, namely:

- (a) 0.6 g. Urea (weakly alkaline)
- (b) 0.6 g. Urea after boiling ( " " )
- (c) Control ( " " )
- (d) 0.6 g. Asparagine ( " " )
- (e) 0.6 g. Urea (no addition of NaOH, weakly acid).

With a sheet of moistened red litmus paper hanging from a cotton plug, they were kept at the room-temperature ( $9^{\circ}\text{C}$ ). After 5 minutes the flask (a) already showed the paper turning blue and a little later also the flask (e), while no change was observed in (b), (c) and (d). Indeed, after 30 minutes the tests with Nessler's reagent gave positive result only for (a) and (e). Determinations<sup>1</sup> of the ammonia liberated gave the following results:

	Duration of digestion, in hours.	Ammonia liberated, g.
(a)	14	0.1622
(b)	14	0.0034
(c)	16	0.0000
(e)	16	0.1603

This experiment proves the existence of a substance which is able to hydrolyse urea and liberate ammonia from it.

The writer next made similar experiments with the resting seeds of soy-bean and also succeeded in proving the presence of the same substance.

Experiment II.—2 g. air dry seeds were finely pulverized in a mortar, macerated with water for one hour and filtered. 100 c.c. of the filtrate was divided into two equal portions, one half (B) was boiled for 15 minutes, the other half (A) not boiled. To each of them was added 0.5 g. urea. The flask (C) served as control. After keeping them for some time at the room-temperature ( $9-10^{\circ}\text{C}$ ) the determination of ammonia was made with the following results:

1. The method used was a qualified vacuum distillation method with finely powdered magnesia usta.



	Intervals, hs.	Ammonia, g.
(A)	15	0.1681
(B)	15	0.0026
(C)	18	0.0000

Thus the presence of a urea-splitting substance was proved beyond doubt in the resting seeds as well as in the seedlings.

Experiment III.—1 g. of air dry powdered seeds of soy-bean was kept at the room temperature with 30 c.c. toluol in an Erlenmeyer's flask. Two such flasks were prepared, to one of which was added 2 c.c. of 30% urea solution, while the second flask served as control. As the moistened red paper of the first flask turned blue after 5 minutes, determinations of the ammonia were made after 17 hours for both flasks. The results were as follows:

(A) With urea	0.1704 g. $\text{NH}_3$
(B) Control	0.0002 g. $\text{NH}_3$

This experiment shows that the urea splitting action of the substance is not due to the living activity of the microbes, that may possibly be present in the seeds. The digestion proceeded equally well in the presence of chloroform instead of toluol, so that it can not be ascribed to putrefactive changes. The writer has also examined the question of bacterial agency in the process. Thus seeds were kept for 30 minutes in a  $1/1000$  solution of mercuric chloride, and were then crushed well in a porcelain mortar, 2 c.c. of 30% urea solution added to it and the whole put in 50 c.c. of sterilized water. The moistened red litmus paper in the flask began to turn blue after 15 minutes ( $10^\circ\text{C}$ ), while at the same time the red color reaction due to ammonia formation was obtained by adding a few drops of phenol pluthalein<sup>2</sup> as an indicator. Next 1 g. of the powdered seeds together with 2 c.c. of 30% urea solution was digested with 50 c.c. of 0.005%  $\text{HgCl}_2$  solution. After 30 minutes, in this case,

2. Phenolphthalein has no injurious action upon the hydrolytic action. As the process goes on naturally very rapidly, the colour reaction can be obtained after a few minutes. This method of using phenolphthalein for demonstrating the presence of an enzyme may be of some value.

the formation of ammonia in the flask was observed, although the action went on somewhat more slowly.

These experiments demonstrate clearly that the hydrolytic process is due to an enzymatic action and not to the living activity of microbes.

Experiment IV.—100 healthy seedlings<sup>22</sup> of soy-bean about 3 cm. high were washed after removing their cotyledons and triturated well in a mortar. To the filtrate was added a mixture of 90% alcohol and ether, whereby a white amorphous precipitate was obtained. This was sucked off after 18 hours, washed and after well pressing with a spatula, extracted with 30 c.c. water. To the extract strong alcohol and ether was again added until precipitation was complete. The precipitate was collected on a Buchner's filter, pressed, washed with alcohol and dried on sulphuric acid. The precipitate thus obtained weighed ca. 0.43 g. and was nearly pure enzyme. It was sparingly soluble in cold water and the aqueous solution gave very weak biuret reaction. The same precipitate was in exactly the same way obtained from the powdered seeds of soy-bean.

0.4 g. of the sample thus prepared was dissolved in 80 c.c. water, with 30 c.c. of which the following experiment was made, in order to ascertain the behavior of the enzyme.

3 flasks of ca. 100 c.c. capacity were filled each with 10 c.c. of the enzyme solution, and to them was added:

- (A) 0.2 g. urea and 30 c.c. water (neutral),
- (B) 0.2 g. urea and 30 c.c. of 1.3% NaOH solution,
- (C) 0.2 g. urea after boiling for 20 minutes.

Each flask was kept with the addition of some toluol in a thermostat at 23° C and ammonia determination was done after a day with the following results:

3. The seedlings used were carefully examined, and in all cases only those which seemed perfectly healthy and normal were employed for the purpose of the experiments.

	Intervals, hs.	Ammonia, g.
(A)	16	0.0671
(B)	17	0.0000 <sup>1</sup>
(C)	14	0.0010

The second experiment with 20 c.c. of the enzyme solution prepared from 5 g. powdered seeds was made in the following manner.

Flask A. ... .. 0.5 g. urea      added (neutral).  
 „ B. ... .. „ „ asparagine „ (0.1% NaOH solution).

After keeping them at 20°C ammonia was determined as follows:

	Intervals, hs.	Ammonia, g.
Flask A.	18	0.1641
„ B.	42	0.0002

These experiments prove beyond doubt that the hydrolysis of urea by soy-bean seeds is principally caused by the action of the enzyme present in them.

Dr. K. Shibata<sup>2</sup> has previously shown that the mycelium of *Aspergillus niger* is able to split urea with liberation of ammonia, but in this case the action is far less energetic than in ours. I shall here extract some figures from his article.

	Ammonia formed, g.	Fungus used, g.	Urea used, g.	Temperature (°C)	Intervals (days).
I	0.0318	0.25	0.75	37	10
II	0.0150	0.50	2.00	35	2
III	0.0306	1.00	1.50	35	9
IV	0.0195	0.50	1.50	20	25
V	0.0174	0.50	0.50	20	28

Experiment V.—The writer considered it interesting to compare the strength of the splitting action of the enzyme in seeds and in seedlings.

4. Both distillation and titration were repeated, but no ammonia could be demonstrated. It has since been found that the enzymatic action is inhibited in such a comparatively high concentration of NaOH as 1%.

5. Hofmeister's Beitr. zur chem. Physiol. u. Pathol. Bd. V, 1904, s. 384.

Unfortunately he can not give exact figures here, since the enzyme in the seedling appears to be somewhat injured by drying. Thus much, however, can be said that the enzymatic action is stronger in the resting seeds than in the seedlings, as may be seen from the following.

(A) 2 g. of air-dried seedlings ca. 3 cm. long, with cotyledons removed, was crushed in a mortar with a little water, the water was then increased to 80 c.c., 30 c.c. of which was decanted and put in a flask. . . . . (a).

(B) To 2 g. of air-dried powdered seeds was added 80 c.c. water, 30 c.c. of which was decanted and put in another flask. . . (b).

These flasks *a* and *b* were prepared in duplicates for the purpose of control. The flasks were treated as follows:

(A) . . . . . (a) alone.

(B) . . . . . (b) alone.

(C) . . . . . to (a) 0.5 g. urea added.

(D) . . . . . to (b) 0.5 g. urea added.

After keeping them at 27°C in the usual manner, ammonia determination was made:

	Intervals, hs.	Ammonia formed, g.
(A)	52	0.0010
(B)	52	0.0024
(C)	14	0.0661
(D)	14	0.2688

Experiment VI.—The action of the enzyme on urea has been thus clarified, but its action on other urea-derivatives remained to be seen. The writer has, therefore, undertaken to examine the following compounds on this point.

(1) Biuret is only slightly attacked by the enzyme. 1 g. of powdered seeds of soy-bean was macerated with water and filtered. The filtrate was divided into 4 equal portions of 20 c.c. each. They were put in flasks and treated as follows:

(A) Boiled and 0.25 g. biuret added,

(B)	Not boiled	"	"	"	"	"
(C)	"	"	"	"	urea	"
(D)	"	"	"	0.5	g. biuret	"

They were kept at the room-temperature ( $8^{\circ}\text{C}$ ) and ammonia was determined as follows:

	Intervals, hs.	Ammonia formed, g.
A	15	0.0013
B	15	0.0050
C	19	0.0874
D	17	0.0056

(2) Both nitrate and oxalate of urea recently prepared were tested for the purpose. They were acted on by the enzyme as well as pure urea, with, however, a slight retardation of liberation of ammonia.

(3) The following compounds were also tested, but they all gave negative results.

Guanidine (carbonate)

Arginine (both nitrate and methylester hydrochloride)

Benzamide,

Allantoin,

Leucine

Alanine

Tyrosine

Kreatine

Histidine (hydrochloride)

Guanine ( " )

Glycocoll (ethylester hydrochloride)

Uric acid

Hippuric acid.

The results obtained thus far plainly show that there is thus one compound—urea—which is acted on by the enzyme with an energetic formation of ammonia, and that biuret is acted on but slightly.

Experiment VII.—Our experiments now assumed a new turn and

a series of tests were made for the behavior of various kinds of plants towards urea. 7 different kinds of seeds were used, and in all cases 50 c.c. of 1% urea solution was mixed with 2 g. powdered seeds in an Erlenmeyer's flask, and kept at the room-temperature. The determination of ammonia gave the following results:

	Intervals, days.	Ammonia, g.
Control . . . . .	3	0.0012
<i>Glycine hispida, maxim</i> . . . . .	1	0.2653
<i>Glycine hispida, forma</i> . . . . .	1	0.2713
<i>Phaseolus vulgaris, forma</i> . . . . .	3	0.0165
<i>Phaseolus radiatus</i> . . . . .	4	0.0037
<i>Pisum sativum</i> . . . . .	5	0.0018
<i>Triticum vulgare</i> . . . . .	4	0.0018
<i>Avena sativa</i> . . . . .	3	0.0165

Thus *Glycine hispida, maxim.* and *Glycine hispida, forma* were seen to be analogous to soy-bean in this regard.

A second series of tests were likewise made with the resting seeds of different plants. In these cases, however, no determination of ammonia was made, as it was too little to be measured. The results were as follows, + indicating a positive and—, a negative result.

Control	—	Maize	—
Barley	—	Rape	—
Upland rice	—	Radish	—
Paddy rice	+	Buckwheat	+
Rye	—	<i>Cucumis melo</i>	+

Thus positive results were obtained only for paddy rice, buckwheat and *cucumis melo*, though the action was but slight.

Experiment VIII.—The writer next tried to localize the enzyme in the seed. For this purpose *Glycine hispida, maxim* was used and phenol phthalein served as the indicator of ammonia formation. The tests proved the presence of the enzyme in all parts of the seed.

Experiment IX.—The range of the enzyme action with regard to the



concentration of urea solution was studied and the result was remarkable. Indeed, with 10, 20 and 30% urea solutions the action went on vigorously, with 40% also well, but with a little retardation.

The influence of foreign substances, such as acids, alkalis, neutral salts, and antiseptics was next observed with the enzymes of soy-bean seeds. The color reaction with phenol phthalein previously mentioned served for the tests which were made after neutralization of the solution. In carrying out the observations the enzyme was submitted to the action of the reagents before being added to the urea solution. During the tests the room-temperature ranged from 13 to 16°C. The results are expressed in the following table.

Foreign subst. used	Concentration	Appearance of red coloration.
$\text{H}_2\text{SO}_4$ .. .. .	1.0 %	after 45 minutes.
" .. .. .	0.5 %	" 15 "
HCl .. .. .	1.0 %	" 40 "
" .. .. .	0.5 %	" 12 "
$\text{H}_3\text{BO}_3$ .. .. .	1.0 %	" 30 "
" .. .. .	0.5 %	" 10 "
NaOH .. .. .	0.5 %	" 35 "
KOH .. .. .	0.5 %	" 30 "
$(\text{NH}_4)_2\text{SO}_4$ .. .. .	5.0 %	" 50 "
" .. .. .	1.0 %	" 10 "
$\text{CuSO}_4$ .. .. .	0.05%	" 55 "
" .. .. .	0.01%	" 10 "
NaF .. .. .	0.25%	" 40 "
" .. .. .	0.20%	" 15 "
$\text{HgCl}_2$ .. .. .	0.05%	" 45 "
" .. .. .	0.02%	" 15 "
$\text{HCOH}$ .. .. .	0.5 %	" 20 "
" .. .. .	0.3 %	" 10 "

Thus 1%  $\text{H}_2\text{SO}_4$ , 1% HCl, 5%  $(\text{NH}_4)_2\text{SO}_4$ , 0.05%  $\text{CuSO}_4$ , 0.25% NaF and 0.05%  $\text{HgCl}_2$  were seen to impede the action of the enzyme to some extent.

Further it was observed that 1% NaOH, 2%  $\text{H}_2\text{SO}_4$ , 10%  $(\text{NH}_4)_2\text{SO}_4$ , 0.1%  $\text{CuSO}_4$ , and 0.1%  $\text{HgCl}_2$  are strongly inhibitory, while MgO, though present in great excess, has no deleterious influence on the action of the urease.

The degree of temperature at which the enzyme is destroyed was next studied. At  $72^\circ\text{C}$  more than one hour were required. Heating for 30 minutes to  $75^\circ\text{C}$  was not sufficient to destroy all the enzyme, but it injured a great part of it. After heating for 5 minutes to  $77^\circ\text{C}$  a faint trace of hydrolysing power was still observed, but no trace was noticeable after heating for one minute to  $80^\circ\text{C}$ . Cooling for one hour to  $-5^\circ\text{C}$  did not decrease the intensity of the hydrolysing power, even at  $-8^\circ\text{C}$  the enzyme still retained its activity for 20 minutes.

The optimum temperature for the working of the enzyme was found to be  $40\text{--}45^\circ\text{C}$ .

Experiment X.—Trypsin, pancreatin, emulsin and Taka-diastrase were tested as to their possible hydrolytic action on urea. Results obtained by adding 0.5 g. of the enzymes to 20 c.c. of 1% urea solution, were entirely negative, as was expected, although they had a powerful action on fibrin, amygdalin and starch respectively.

The necessary preliminary studies being finished the writer proceeded to determine the value of the enzyme when applied to human urine. It has been observed by various authors that urea, which is the chief nitrogenous constituent of fresh urine, is not absorbed and fixed by the soil as the ammonium compounds are, and that it is even injurious to plant roots in a comparatively high dilution (0.5 per mille)<sup>6</sup>. Fresh urine is, therefore, subjected to a process of fermentation in order to secure the nitrogen in an available form. The loss of ammonia during the process has been a great disadvantage, and various plans have been devised for saving the valuable nitrogen.

In spite of the indefatigable zeal of agricultural chemists in this

6. Cf. Bul. of the College of Agr., Tokyo. Vol. IV. 413.

direction no efficient contrivance has been found out. The application of the new enzyme for this purpose naturally occurred to me, and led to the desired result.

Experiment XI.—200 c.c. fresh urine of a healthy man was collected and to 100 c.c. of it was added 1 gram of powdered soy-bean seeds, while the other 100 c.c. served as control. With loosely tightened cotton plugs, they were left in the room (temperature ranged  $8-10^{\circ}\text{C}.$ ) for 16 days. The mixture was then taken out of the flasks and the ammonia formed was determined with the following results:

(A) With seeds . . . . .	0.358 g. $\text{NH}_3$ .
(B) Control . . . . .	0.036 g. $\text{NH}_3$ .

This shows that 0.322 g. more ammonia was produced by the addition of the seeds. Similar experiments also showed that the alkaline fermentation of urine was favoured by the addition of the seeds, as the following figures will show:  $\text{NH}_3$  formed, g.

	$8-9^{\circ}\text{C. 10 days}$	$8-9^{\circ}\text{C. 1 day.}$
(A) . . . . .	0.362	0.173
(B) . . . . .	0.042	0.019

It may safely be said that the enzyme, when applied to fresh urine, produces 4.5 g. more ammonia from 1 L. urine in a day even in colder seasons.

Experiment XII.—300 c.c. of fresh urine collected in the morning was mixed with several grams of powdered soy-bean seeds in an Erlenmeyer's flask and kept at the room-temperature ( $8-9^{\circ}\text{C}.$ ) with a cotton plug. Ammonia determination after several days gave the following results. (Calculated in terms of 100 c.c. urine.)

Seeds used	Intervals, hs.	Temp.	Ammonia, g.
Control . . . . .	144	$8-9$	0.016
0.1 g. . . . .	18	9	0.304
0.5 g. . . . .	77	$8-9$	0.127
1.0 g. . . . .	7	9	0.675
2.0 g. * . . . .	91	$8-9$	0.705

7. (B) is the control, without the addition of seeds.

3.0 g.	.. .. .	91	8-9	0.726
5.0 g.	.. .. .	3	9	0.313
5.0 g.	.. .. .	96	8-9	0.726
10.0 g.	.. .. .	140	8-9	0.687
5.0 g. (no urine)	.. .. .	140	8-9	0.001
5.0 g. (boiled)	.. .. .	144	8-9	0.031

The percentage of total nitrogen in the urine was found after Kjeldahl's method to be 0.814. It is easily seen that the most favorable ratio of seeds to urine lies between 3:300 or 1:100, and 5:300 or 1.7:100; where 0.726 g. ammonia was produced in only 4 days from 100 c.c. urine.

It may also be concluded that when properly managed, almost all urea-nitrogen of urine can be changed into a form of ammonia in a few days. Hence, it is advisable that preservative agents, *e.g.* sulphuric acid &c. be added at the proper moment to fix the ammonia produced; or if ammonium sulphate may be manufactured on a large scale from the hydrolysed urine.

The writer was next led to step into the biological application of the enzyme for analytical purposes.

Experiment XIII.—10 g. of pulverized soy-bean seeds was macerated in cold water for 30 minutes, the filtrate obtained was divided into 5 equal parts of 10 c.c. each and urea was added as follows:

- (A) 0.01 g. urea dissolved in 90 c.c. water=0.01 %  
 (B) 0.005 „ „ „ „ „ „ „ „ =0.005%  
 (C) 0.002 „ „ „ „ „ „ „ „ =0.002%  
 (D) 0.001 „ „ „ „ „ „ „ „ =0.001%

Naturally at the beginning of the experiment every flask showed an acid reaction, as proved by the addition of phenol phthalein. The room-temperature during the test was 11°C throughout. The red colour appeared in (A) after 20 minutes, in (B) after 30 minutes and in (C) after 45 minutes, while in (D) it did not appear.

This shows that the enzyme is able to split urea in even  $1/50000$  solution to a recognizable extent, when neutral. The enzyme can there-

fore be satisfactorily employed for the urea-test of various animal juices, and further improvements will probably make quantitative determination of the urea possible. This point is reserved for future study.

## Conclusions

Since *Musculus*<sup>1</sup> discovered a urea-splitting enzyme in the urine which he obtained from a patient, the behavior of urease became the subject of earnest investigations by various authors. *Lea*<sup>2</sup> concluded that its action was altogether intracellular and that it was unable to pass out of the cell during life. *Miquel*,<sup>3</sup> on the other hand, claimed that a powerful urease could be obtained quite free from bacterial cells. *Beijerinck*<sup>4</sup> and *Moll*<sup>5</sup> after exhaustive series of experiments came to the conclusion that the urease in *Micrococcus ureae* was by no means detached from the cells whether the bacteria were alive or not. These conflicting statements need to be cleared up. My present investigation has demonstrated that urease exists not only in lower organisms, but also in higher plants, and that the urease in the latter in which it acts more powerfully can be extracted with water very easily.

It is strange that the urease acts exclusively on urea and not on allied substances. Its natural function in the plant body has still to be made out.

There is no doubt that the enzyme is important, and its urea-splitting property can be turned to account for determining the presence of urea even in minute quantities in various organs and juices.

Another application of the enzyme consists in its strong ammonifying action on fresh urine, and its application for the recovery of the chief

1. *Musculus*, *Compt. rend.* 82, 1876, 334.

2. S. Lea, *Journ. of Physiol.* Vol. 11, 1890.

3. P. Miquel, *Compt. rend.* 111, 1890, 397.

4. Beijerinck, *Centrbl. f. Bakt.* II. Abt. VII, 1901, 33.

5. Moll, *Beitr. z. chem. Physiol. u. Patholog.*, 1902, II, 344.

nitrogen of fresh urine is undoubtedly a step in advance in the economy of manures.

I have to express to Professor Dr. *U. Suzuki* my great indebtedness for his advice so kindly given during the progress of the work.

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On the Existence of an Enzyme in the Silkworm, which  
produces Ammonia as a Cleavage Product  
of Amino-Compounds.

BY

T. Takeuchi and R. Inoue.

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While engaged in an investigation of the chemical constituents of the silkworm (*Bombyx mori*) at different stages of its life-cycle, especially the mature worm, pupa and moth, at the suggestion of Professor U. Suzuki, we found a new enzyme of which a general description is given below.

I. *The action of the juice of crushed silkworms on some amino-compounds.*

Ten worms were well crushed in a porcelain mortar, and divided equally into four Erlenmeyer's flasks, and to each was added 100 c.c. of distilled water. They were then treated as follows:

- (1). No reagents.
  - (2). 0.5 gm. of asparagine.
  - (3). A little quantity of leucine and glycocoll.
  - (4). The same quantity of leucine and glycocoll as in the third flask, and enough caustic soda to make the solution 0.1%.
- (The juice of the crushed worms gives a weakly alkaline reaction).

As an antiseptic enough toluole was poured into each flask to cover the surface of the solution, the flasks were then closed with cotton stoppers, and kept at 25°C in a thermostat. After standing for 24 hours, a small quantity of each solution was filtered into a test tube, and tested for ammonia with Nessler's solution, with the following results:

The filtrate from the second flask gave a strong reaction for ammonia, while for the others the reaction was very weak. In particular,

the fourth remained entirely without the color on the addition of *Nessler's* solution.

This preliminary experiment appears to show the presence of an enzyme in the juice of crushed silkworms. Similar experiment with some pupæ, and moths, not only gave similar results, but the reaction for ammonia was even stronger than in the larvæ.

The next experiment was a step forward. Ten moths were well crushed in a porcelain mortar, and transferred into two flasks of the capacity of some 200 c.c. each, and diluted with a small quantity of water; to each was then added one half gram of asparagine, and one of them was boiled. Toluole was used as an antiseptic, and the flasks were provided with cotton stoppers, as in the previous experiment. The flasks were then kept at 25°C. for 24 hours. Then a small quantity of the two solutions was filtered and tested for ammonia by the same method as before. The filtrate of the unboiled solution gave a strong ammonia reaction, while the others gave none at all. After keeping them for one week at the same temperature, the nitrogen of the solution in the form of ammonia was determined with the following results:

Boiled . . . . .	0.0009 gm.
Unboiled . . . . .	0.0456 ..

The determination of the ammonia nitrogen was done by the following method.

The solution was poured into a distiller provided with a side tube, into which magnesia usta was dropped in small quantities by means of a spoon, the distiller being at the same time shaken violently so as to ensure an uniform distribution of the salt, until the solution presented a faintly alkaline reaction. Then the distiller was connected with a receiver, also provided with a side tube, through which the air was sucked out. The distillation thus took place under a decreased pressure, until all the ammonia was liberated from the solution. The ammonia was fixed by the standard solution of sulphuric acid which had been previously put in the receiver. When the distillation was complete, volumetric determination of the nitrogen was made.

## II. *Isolation of the enzyme.*

The existence of an enzyme in silkworm being established, our next task was to isolate it. For this purpose, one hundred moths were thoroughly crushed, mixed with some clean sand, in a porcelain mortar. The mass transferred into a well washed cotton cloth, and the juice squeezed out. The refuse was also pressed thrice in the same way. The juice thus obtained amounted to some 100 c.c. On addition to it a mixture of absolute alcohol and a little ether enough to make 600 c.c., it yielded a grayish-white bulky precipitate. The solution was allowed to stand for about 16 hours until the precipitate had settled down on the bottom of the flask, and then filtered. The precipitate thus obtained weighed five grams when freed from the alcohol as much as possible. It is soluble in water and shows the weak biuret reaction.

In order to test the presence of the enzyme in the precipitate, the following experiment was carried on.

One half of the precipitate weighing 2.5 grms., was dissolved in 25 c.c. of water, and equally divided into five flasks, and treated as follows:

- (a) Diluted with water to 40 c.c., served as control.
- (b) Also diluted with water to 40 c.c. with addition of 0.4 grm. of asparagine.
- (c) Boiled, and then treated in the same way as (b).
- (d) Diluted with water to 40 c.c. with addition of 0.4 grm. of asparagine and enough caustic soda to make the strength of 0.05 per cent.
- (e) Boiled, and then treated just like (d).

The flasks, with a little quantity of toluole, were closed with cotton stoppers and kept at 28°C. for three days. Then the filtrate of the five solutions were examined one by one with *Nessler's* reagent. The result was as follows:—The filtrate of the flask (d) showed a strong ammonia reaction, while the others showed none. This shows clearly that in the case of asparagine the enzyme acts only in a faintly alkaline solution, and that it is inactive or nearly so even when the solution is neutral. After five days' standing, the ammonia produced in

(d) and (e) was determined by the method described before, with the following results.

	(d)	(e)
Nitrogen as ammonia . . .	0.0062 grm.	0.

The action of the enzyme was precisely the same after purification.

### III. *The action of the enzyme on various amino-compounds.*

To ascertain whether the enzyme acts on other amino-compounds besides asparagine, the following experiment was made.

Ten moths were well crushed in a mortar with a little water added, transferred into a flask of the capacity of some 200 c.c., and then diluted with water to 50 c.c. Several such flasks were prepared and various amino-compounds were added in the proportion of one per cent. They were then well shaken with enough toluole as an antiseptic and kept at 28°C. for six days, with occasional shaking so as to well mix the toluole and the solution to prevent putrefaction. After one week the ammonia in each flask was determined by the previous method, with the following results:

Amino-compounds.	Nitrogen as ammonia.
Asparagine . . . . .	0.0179 grm.
Urea . . . . .	0.0051 ..
Buret . . . . .	0.0051 ..
Leucine . . . . .	0.0040 ..
Glycocoll . . . . .	0.0038 ..
Tyrosine . . . . .	0.0027 ..
Allantoin . . . . .	0.0027 ..
Guanidine carbonate . . . . .	0.0022 ..
Benzamide . . . . .	0.0022 ..
Control, without any addition . . . . .	0.0022 ..

This shows that the enzyme acts chiefly on asparagine, the other amino-compounds being affected very slightly or not at all.

### IV. *The cleavage products of asparagine.*

We now come to the determination of the cleavage products of asparagine. For this purpose, one-half gram of the precipitated enzyme

was dissolved in 50 c.c. of water, and 0.025 gram of asparagine added. After treating the flask containing the solution as before, it was kept at the room-temperature (about 5°C) for two weeks, then the solution was filtered and the filtrate transferred into a separating funnel, acidified with 5 c.c. of 20 per cent sulphuric acid. Then the solution was extracted with ether several times until the ethereal extract presented a very faintly acid reaction. All the extracts were combined together and slowly evaporated on a water bath. White prismatic crystals were thus obtained, which are perhaps those of succinic acid. But the sample was too small in quantity to be used for further researches.

#### V. Conclusions.

The presence in the silkworm, and especially in the moth, of an enzyme which produces ammonia by acting on asparagine has been placed beyond doubt. Similar enzymes have been reported by many authors, and it may not be useless to give a brief review of their works.

K. Shibata<sup>1</sup> has ascertained the presence of an enzyme which produces ammonia by splitting urea, biuret, acetamide, and two or three other amino-compounds, in the filaments or mycelium of *Aspergillus niger*. But this enzyme differs from ours in that it acts chiefly on urea, and one or two other amino-compounds but very weakly on asparagine. Moreover, as the enzyme was not isolated, the development of ammonia in this case may have been due to the combined actions of several enzymes instead of one. E. Castoro<sup>2</sup> observed the production of ammonia during the germination of one or two species of *Lupinus*, kept at 25°—30°C. for two weeks with some toluole as an antiseptic, but he has not effected the isolation of the enzyme. Again M. Jacoby<sup>3</sup> has ascertained that ammonia is produced by the autolysis of the dog liver but he attributes the phenomena to the action of a proteolytic enzyme and has not determined its exact nature.

1. Beiträge zur ch. Physiol. u. Pathol. 1904, Bd. 5, S. 384.

2. Z. f. physiol. Ch. 1907, Bd. 50, S. 525.

3. Z. f. physiol. Ch. 1900, Bd. 30, S. 149.

Lately, *Effront*<sup>4</sup> has discovered an enzyme which produces ammonia by splitting asparagine, in the beer yeast, but according to his observations the enzyme acts most favorably in a strongly alkaline solution (0.1—0.2%), and not at all in neutral or weakly acid solutions, which distinguishes it from our enzyme. Lastly, *E. Weinland*<sup>5</sup> has observed that the fly *Calliphora Vomitoria* produces ammonia during the larval stage, and has attributed it to the action of an enzyme. We have also tried to repeat the experiment on the silkworm, larva and moth, but no positive result was obtained.

In short, an enzyme, which produces ammonia by splitting amino-compounds, may be found widely distributed in plants and animals, but owing to want of observation in a pure state, it is not possible to determine whether the action is due in each particular case to one or several enzymes. We have prepared our enzyme in a comparatively pure state, and there is no doubt that it is different from any as yet described. What rôle it plays in the metamorphosis of the silkworm is totally unknown.

*Addendum.*—Since the above was communicated to the Tokyo Chemical Society, Dr. *Bulkewitsch* has published “Das Ammoniak als Umwandlungsprodukt stickstoffhaltiger Stoffe in höheren Pflanzen.” (*Biochemie Zeitschr.* Bd. 16. s. 411).

4. *Compt. rend.*, 1908, 146, 779.

5. *Z. f. Biol.*, 1905, Bd. 47, 186.

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# Ueber die Extraktivstoffe im Fischfleiſche.

VON

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unter Mitwirkung von M. Yamakawa und Y. Irie.

## EINLEITUNG.

Unsere Kenntnis über die Extraktivstoffe der tierischen Muskeln ist in letzter Zeit durch Untersuchungen von Gulewitsch, Krimberg<sup>1</sup>, Kutscher<sup>2</sup>, Ackermann<sup>3</sup>, Micko<sup>4</sup> u. A. bedeutend erweitert worden. Diese Autoren beschäftigten sich hauptsächlich mit dem Liebig'schen Fleischextrakt und haben ausser den altbekannten Stoffen: Xanthin, Hypoxanthin, Kreatin, Kreatinin, Taurin, Guanin, Adenin etc. das Vorhandensein von Carnosin, Karnin, Karnitin, Oblitin, Neosin, Karnomuskalin, Methylguanidin etc. und auch von kleinen Mengen der Monaminoſäuren, wie Alanin und Glutaminsäure nachgewiesen.

So wird das physiologisch hochwichtige Problem der chemischen Natur der Extraktivstoffe, ihre Bildung und Umwandlung, ihre Bedeutung für den gesamten Kreislauf im tierischen Organismus und ferner die

1. W. I. Gulewitsch, Krimberg u. Amiradzibi: Zur Kenntnis der Extraktivstoffe der Muskeln I—X. Mitteilung:—Zeitsch. f. physiol. Chem. 30 565 45 326. 47 471 48 412 49 89 50 204 361 535. 53 514 55 466 56 417.

2. Fr. Kutscher: Zur Kenntnis des Novains:—Zeitsch. f. physiol. Chem. 49 47. 484  
„ Zweite Notiz zur Kenntnis des Novains „ „ 50. 250.

3. D. Ackermann u. Fr. Kutscher: Zur Konstitutions ermittlung des Novains: Zeitsch. f. physiol. Chem. 56 220.

D. Ackermann: Ein Beitrag zur Chemie der Faulnis: Zeitsch. f. physiol. Chem. 57 1.  
„ „ Ein Faulnis versuch mit Arginin „ „ 50 305.

4. K. Micko: Ueber das Vorkommen von Monaminoſäuren im Fleischextrakt „ 50 180.

physiologische Wirkung derselben als menschliche Nahrung immer mehr in helleres Licht gerückt werden.

Was nun das Fischfleisch betrifft, so fehlt es darüber an chemischen Studien nicht; diese befassen sich aber meistens mit den Fäulnisprodukten desselben. Auf die normalen Bestandteile dagegen hat man nur selten die Aufmerksamkeit gerichtet.

Da der Fisch in Japan als Volksnahrungsmittel eine ebenso wichtige Stelle einnimmt, wie das Fleisch in Europa, so ist es für uns Japanern ganz besonders wünschenswert, die chemischen und physiologischen Studien in dieser Richtung weiter vorwärts zu bringen. Ferner steht die Erscheinung der Fischvergiftung, die bei uns so oft vorkommt, mit den Extraktivstoffen in innigstem Zusammenhang; nur durch gründliche Studien der Natur der letzteren kann dieselbe erklärt werden.

Von diesen Grundgedanken ausgehend haben wir unsere Studien begonnen und legen die bisherigen Ergebnisse der Öffentlichkeit vor. Bis jetzt haben wir so wohl im frischen wie im getrockneten Fleisch von *Katsuo* (Bonito, *Gymnosarda pelamis*), *Lachs* (*Onchorynelmus keta*), *Maguro* (*Thynnus thynnus*), *Ise-ebi* (Hummer, *Panulirus* sp.), *Ika*<sup>1</sup> (*Ommastrephes* sp.) und *Unagi* (Süßwasser-Aal—*Anguilla fluvialis*) ausser den bisher bekannten Stoffen, wie Kreatin, Kreatinin, Xanthin, Hypoxanthin, Taurin etc. das Vorkommen von *Carnosin*, *Histidin*, *Arginin*,<sup>2</sup> *Lysin* und *δ-Aminovaleriansäure* in ziemlich bedeutender Menge konstatieren können. Ferner konnten wir verschiedene Monaminosäuren, wie *Tyrosin*, *Leucin*, *Alanin*, *Prolin* etc. mit verhältnismässiger Leichtigkeit nachweisen, Stoffe die man aus dem Liebig'schen Fleisch extrakt nur in unbedeutender Menge zu isolieren vermochte.

Wir halten es für wahrscheinlich, dass man schliesslich fast alle Spaltungsprodukte der Eiweisskörper, nebst deren Umwandlungspro-

1. Obgleich *Ise-ebi* u. *Ika*, eigentlich nicht zu den Fischarten gehören, so haben wir doch der Bequemlichkeit halber hier angereiht, weil sie als menschliche Nahrung ebenso wichtig sind wie der Fisch.

2. Das freie Arginin ist nur einmal in der Milz gefunden worden: Vergl. W. I. Gulewitsch u. Joelhelson:—Zeitsch. f. physiol. Chem. 30 533.

dukten in den tierischen Muskeln finden wird, ebenso wie dies in der Pflanze der Fall ist. Wir sind auch zu der Annahme geneigt, dass man diese Extraktivstoffe nicht als Ausscheidungsstoffe wie z. B. Harnstoff betrachten darf, sondern als eine Körpergruppe, die für den weiteren Kreislauf im tierischen Organismus eine wichtige Rolle spielt. Besonders interessant ist das reichliche Vorkommen von Hexon basen in Fisch-(und Hummer) muskeln, was vielleicht mit der Bildung von Sperma und Eiern in gewissem Zusammenhang stehen dürfte, da die letzteren ausserordentlich reich an basischen Stoffen sind.

Dass die giftigen Basen wie Ornithin, Putrescin, Cadaverin etc. durch Fäulnis von Arginin und Lysin gebildet werden, ist von Ellinger, Ackermann u. A. nachgewiesen worden. Was aber das Histidin und Carnosin betrifft, müssen wir noch die Ergebnisse künftiger Forschungen abwarten. Eine Base, die wir im getrockneten *Ika* in reichlicher Menge gefunden haben und für *o*-Aminovaleriansäure halten, ist noch niemals in frischen Muskeln gefunden worden. Es sei nur erwähnt, dass der letzt genannte Körper von Salkowski<sup>1</sup> aus gefaultem Pankreas isoliert und später von D. Ackermann<sup>2</sup> näher studiert worden ist. Kurzum, es bleibt in dieser Richtung noch viel zu tun übrig. Wir beabsichtigen daher diese Arbeit noch weiter fortzusetzen und hoffen etwas neues in diesem Gebiete beitragen zu können.

In den folgenden Seiten teilen wir die einzelnen Ergebnisse der bisherigen Untersuchung mit.

## I. KATSUO (*Gymnosarda peltamis*, od. *Bonito*).

### A. Katsuobushi (Getrockneter Bonito).

Die von uns untersuchte Katsuobushi Probe hatte folgende quantitative Zusammensetzung:

1. E. u. H. Salkowski:—Berichte d. deutsch. chem. Gesellschaft, XVI. 1191 u. XXXI. s. 776.

2. D. Ackermann: Ein Beitrag zur chemie der Fäulnis: Zeitsch. f. physiol. Chem. 54 1.

„ „ „ Ein Fäulnisversuch mit Arginin: „ „ „ 56 395.

## In 100 Teilen Trocken substanz.

Gesamt Phosphor	1.469		
In Wasser löslicher Phosphor	1.211		
Gesamt Stickstoff	14.611		
In Wasser löslicher Stickstoff	3.350		
Darunter :	{	Ammoniak stickstoff	0.072
	{	Eiweiss stickstoff	0.176
	{	Nicht-Eiweiss stickstoff	3.102
	{	Durch Phosphowolframsäure fällbarer Stickstoff	1.289
	{	(Ammoniak ausgenommen).	

## In Wasser löslicher Stickstoff als 100.

Ammoniak stickstoff'	2.15
Eiweiss stickstoff'	5.25
Nicht-Eiweiss stickstoff'	92.60
Darunter :	{
Durch Phosphowolframsäure fällbarer Stickstoff'	38.21
Durch Phosphowolframsäure nicht fällbarer Stickstoff'	54.39

## Unter dem durch Phosphowolframsäure

fällbaren Stickstoff: Derselbe als 100

{	Durch Silber nitrat in neutraler Lösung fällbarer Stickstoff	0.067	5.24
{	Durch Silbernitrat und Baryt fällbarer Stickstoff	1.008	78.75
{	Stickstoff in anderer Form	0.205	16.01

*Isolierung der organischen Basen.*

1 Kg getrockneter und fein gepulverter Bonito wurde mit Wasser eine Stunde gekocht und stark abgepresst. Der Rückstand wurde noch zweimal in derselben Weise behandelt. Die vereinigten Auszüge wurden mit Essigsäure schwach angesäuert und mit einer wässrigen Tannin

Lösung versetzt, wobei ein dicker Niederschlag entstand. Das Filtrat wurde durch Bleiessig von Tannin und anderen Verunreinigungen befreit, abfiltriert und nach dem Entfernen des Bleies durch Schwefelsäure, wurde es mit Schwefelsäure angesäuert bis die Flüssigkeit ungefähr 5% derselben enthielt und mit einer conc. Lösung von Phosphorwolframsäure gefällt. Der dabei in reichlicher Menge entstandene weisse Niederschlag wurde nach 24 Stunden abgesaugt, mit 5% Schwefelsäure gewaschen und auf der Tonplatte getrocknet. Der Niederschlag wurde nun in wenig Wasser verteilt, mit Ueberschuss von Barium hydroxid verrieben. Das Gemisch wurde öfters umgerührt und bei einer Temperatur von 25-30° 24 Stunden stehen gelassen und abgesaugt. Der Rückstand wurde nochmals in Wasser verteilt und mit Baryt verrieben. Diese Operation wurde dreimal wiederholt. Die vereinigte Filtrate wurden durch Kohlensäure von Baryt befreit und im Vacuum bis auf ungefähr 200 c.c. eingedampft, mit Salpetersäure neutralisiert und mit Silbernitrat lösung in kleinem Ueberschuss versetzt.

I.—Der Silbernitrat-Niederschlag (*Xanthin und Hypoxanthin*). Der Silbernitrat-Niederschlag wurde mit Ueberschuss von Ammoniak verrieben und 24 Stunden stehen gelassen, um damit die Silbernitrat salze der Basen in die Silbersalze derselben überzuführen. Die Silbersalze wurden dann mit warmer verdünnter Salzsäure zerlegt und heiss filtriert. Das Filtrat wurde stark eingeeengt, mit Ueberschuss von Ammoniak versetzt, und 24 Stunden stehen gelassen. Da aber nichts dabei ausgeschieden war (*Guanin* nicht vorhanden) wurde es mit Salzsäure angesäuert, wiederholt zum Trocknen verdampft und zuletzt mit Alkohol verrieben. Der dabei umgelöst gebliebene Rückstand wurde in Wasser gelöst und mit einer ammoniakalischen Silberlösung versetzt. Es entstand dabei eine weisse Fällung von Xanthin silber. Der Niederschlag von Xanthin silber wurde mit Salzsäure verrieben, vom ausgeschiedenen Silberchlorid abfiltriert, zum Syrup verdampft und mit absolutem Alkohol behandelt, um den überschuss von Salzsäure zu entfernen. Der Rückstand wurde mit Wasser versetzt und bei einer Temperatur von 40° 24 Stunden stehen gelassen. Ein Teil ging dabei in Lösung und hinterliess ein schwach gelblich gefärbtes

Pulver, das in kaltem Wasser schwer, in heissem Wasser etwas leichter und in Ammoniak leicht löslich war. Es gibt die Weidels'sche, so wie die Strecker'sche Reaktion, welche eigentlich für Xanthin charakteristisch sind. Leider reichte die Menge der isolierten Base zur näheren Untersuchung nicht aus.

Die Mutterlauge von Xanthin lieferte nach weiterem Eindünnen 0.74 g. Hypoxanthin. Es war in kaltem Wasser und Alkohol schwer, in heissem Wasser aber leicht löslich. Die wässrige Lösung reagierte neutral. Für die Analyse wurde das gereinigte Präparat im Vacuum bei 100° getrocknet.

0.1344 g Subst. gab

0.0384 g Pt

Pt

$(C_5H_4N_4O \cdot HCl)_2 \cdot PtCl_4$

Ber. 28.55

Gef. 28.57

II.—Durch Silbernitrat und Baryt fällbare Base (*Histidin*). Das Filtrat vom Silbernitrat-Niederschlag (I) wurde nun mit Ueberschuss von Silber nitrat und conc. Bariumhydroxid Lösung versetzt, wobei ein dunkelbrauner Niederschlag in reichlicher Menge entstand, der abgesaugt, mehrereremal mit Wasser gewaschen, in Wasser verteilt, und mit Schwefelwasserstoff zerlegt wurde. Das Filtrat vom Schwefelsilber wurde im Vacuum eingedampft; als der Schwefelwasserstoff vollständig von der Flüssigkeit ausgetrieben war, wurde diese mit Schwefelsäure angesäuert und mit einer conc. Lösung von Phosphowolframsäure gefällt. Der Phosphowolframsäure-Niederschlag wurde wieder in oben angegebener Weise mit Baryt zerlegt und weiter verarbeitet. Aus der so gewonnenen stark alkalischen Flüssigkeit, die freie Base enthielt, schieden sich nach mehreren Tage im Exikator allmählich die farblosen Krystalle aus, die ungefähr 15 g. betrugen. Diese Krystalle wurden in Wasser gelöst, mit Kohlensäure gesättigt und mit einer wässrigen Quecksilberchlorid lösung gefällt. Der weisse Niederschlag wurde mit Schwefelwasserstoff zerlegt, vom Quecksilbersulfid abfiltriert und im Vacuum verdampft, um Schwefelwasserstoff auszutreiben, wieder mit Schwefelsäure angesäuert



und nochmals mit Phosphowolframsäure gefällt. Die nach Zerlegung des Phosphowolframsäure-Niederschlags in bekannter Weise erhaltene freie Base war nunmehr fast rein und bestand aus farblosen dünnen Plättchen, die von der Seite betrachtet als dünne Nadeln erschienen. Diese Base war in Aether und Alkohol schwer, in Wasser aber leicht löslich. Die wässrige Lösung reagierte ziemlich stark alkalisch. Sie gab schöne rote Färbung mit einer alkalischen Lösung von Diazobenzolsulfosäure (Pauly'sche Reaktion), gab auch Biuretreaktion beim Erwärmen. Im Kapillarrohr erhitzt zersetzte sie sich bei 237-242° (uncorr.)

Für die Analyse wurde das sorgfältig gereinigte Präparat im Vacuum bei 100° getrocknet.

0.1340 g Subst.	0.0367 g N			
0.1360 g „	0.2300 g CO <sub>2</sub>	0.0783 g H <sub>2</sub> O		
	C	H	N	
C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>	Ber.	46.45	5.81	27.10
	Gef.	46.12	6.40	27.38

Es wurde das methylester salzsaure Salz der Base nach der Vorschrift von E. Fischer und U. Suzuki<sup>1</sup> dargestellt. Es bestand aus farblosen Prismen. Im Kapillarrohr rasch erhitzt, schmolz es bei 195-200 (uncorr.) Für die Analyse wurde es im Vacuum bei 100° getrocknet.

0.1530 g Subst.	0.0272 g N		
0.1412 g „	0.0423 g Cl		
	N	Cl	
C <sub>6</sub> H <sub>8</sub> N <sub>3</sub> O <sub>2</sub> (CH <sub>3</sub> ). 2HCl	Ber.	17.36	29.34
	Gef.	17.78	28.95

Das Pikrat:—Gelbe Prismen, leicht löslich in warmem Wasser, enthält ein Molekül Krytallwasser, das bei 80° verloren geht. Das wasser freie Salz schmilzt erst über 260°C. Für die Analyse wurde es im Vacuum bei 100° getrocknet.

1. E. Fischer und U. Suzuki: Berichte d. deutsch. chem. Gesellschaft. XXXVIII. Band III. 4184.

0.3111 g Subst. gab	0.1879 g Pikrinsäure
0.1052 g „	0.0226 g N
	N Pikrinsäure
$C_6H_9N_3O_2$ $C_6H_3N_3O_7$	Ber. 21.87 59.37
	Gef. 21.47 60.40

Nach den oben angegebenen Daten kann man kaum zweifeln, dass die Base Histidin war.

III.—Das Filtrat vom Silbernitrat und Baryt-Niederschlag (II). (*Carnosin*).

Das Filtrat vom Silbernitrat und Baryt-Niederschlag (II) wurde durch Salzsäure von Silber und durch Schwefelsäure von Baryt befreit, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Aus dem Phosphowolframsäure-Niederschlag wurde in bekannter Weise eine stark alkalische Flüssigkeit, die freie Base enthielt, erhalten. Nach dem diese Flüssigkeit im Vacuum exikator langsam eingedunstet und mit absolutem Alkohol versetzt wurde, schieden farblose prismatische Krystalle allmählich aus, die abgesaugt, in wenig heissem Wasser gelöst und durch Zusatz von absolutem Alkohol umkrystallisiert wurden. Die Ausbeute betrug 3.6 g.

Diese Base war in absolutem Alkohol schwer, in Wasser aber leichter löslich. Die wässrige Lösung reagierte stark alkalisch. Im Kapillarröhr erhitzt, zersetzte sie sich bei 233—235° (uncorr.) Für die Analyse wurde das gereinigte Präparat im Vacuum bei 100° getrocknet.

0.1173 g Subst. gab	0.0291 g N
	N
$C_9H_{14}N_4N_3$	Ber. 24.78
	Gef. 24.82

Es wurde das Platin chloriddoppelsalz der Base dargestellt, indem die freie Base zuerst mit verdünnter Salzsäure neutralisiert und mit einer wässrigen Platin chloridlösung in kleinem Ueberschuss versetzt und langsam verdampft wurde. Es schieden sich dabei unregelmässige Krystalle aus, die im Kapillarröhr erhitzt bei 219—221° (uncorr.) sich

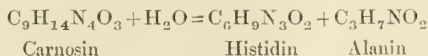
zersetzten. Für die Analyse wurde das Salz im Vacuum bei 100° getrocknet.

0.2604 g Subst		0.02087 g N
0.2262 g „		0.0682 g Pt
		N Pt
$C_9H_{14}N_4O_3 \cdot 2HCl \cdot PtCl_4$	Ber.	8.81 30.63
	Gef.	8.01 30.15

Die Analysen stimmen also mit der Formel  $C_9H_{14}N_4O_3$ , viz. dem Carnosin aus dem Liebig'schen Fleischextrakt. Nach den kleinen Unterschieden in der Löslichkeit und den anderen Eigenschaften zu urteilen, handelt es sich wahrscheinlich um eine Isomerie desselben. Darauf wollen wir später noch zurückkommen.

## B. Das frische Bonito fleisch.

Das Histidin, das wir im getrockneten Bonito in reichlicher Menge gefunden haben, ist ein Spaltungs produkt der Eiweiss körper, besonders des Haemoglobins und bisher niemals in freiem Zustande im tierischen Gewebe konstatiert worden. Im Pflanzenreich scheint es jedoch ziemlich weit verbreitet zu sein, so hat E. Schulze<sup>1</sup> in etiolirten Keimlingen verschiedener Pflanzenarten das Vorkommen von Hexon-basen, viz. Arginin, Lysin und Histidin nachgewiesen. Nach neueren Untersuchungen von W. L. Gulewitsch<sup>2</sup> wird das im Liebig'schen Fleisch extrakt gefundene Carnosin durch Hydrolyse in Histidin und Alanin gespalten.



So könnte man natürlich vermuten, dass das Histidin erst während des Trocknens aus anderen Verbindungen durch enzymatische Spaltung gebildet wird. Um diese Frage zu entscheiden haben wir frisches Bonito-fleisch untersucht und bestätigen können, dass das Histidin tatsächlich

1. E. Schulze:—Hoppe-Seylers' Zeitsch. f. physiol. Chem. 17. 597.

2. W. L. Gulewitsch:—Hoppe-Seylers' Zeitsch. f. physiol. Chem. 50. 535.

in freiem Zustande im Fleisch-gewebe vorhanden und nicht als sekundäres Produkt zu betrachten ist. Zu diesem Zwecke wurden 5 kilo frisches Bonito fleisch fein zerhackt und wiederholt mit heissem Wasser extrahiert. Die gesanten Auszüge wurden, wie oben erwähnt, mit Tannin und Bleiessig gefällt; das Filtrat davon wurde nach dem Entfernen des Bleies durch Schwefelsäure, im Vacuum zu einem Syrup verdampft, mit Alkohol versetzt und im Exikator stehen gelassen. Die zuerst ausgeschiedenen krystalle bestanden hauptsächlich aus kreatin. Die Ausbeute desselben betrug etwa 5 g.

Das Rohprodukt wurde aus heissem Wasser umkrystallisiert und im Vacuum bei 100° getrocknet und analysiert.

0.1696 g Subst.		0.0550 g N
		N
$C_4H_9N_3O_2$	Ber.	32.06
	Gef.	32.43

Das Pikrat des Kreatins bestand aus kleinen gelben Nadeln, die in kaltem Wasser nicht leicht, in heissem Wasser und Alkohol aber leicht löslich waren. Im Kapillar rohr erhitzt zersetzt es sich bei 212—213° (uncorr).

Die Mutter lauge von Kreatin wurde mit Wasser verdünnt, Kohlensäure bis zur Sättigung eingeleitet und mit so viel wässriger Quecksilberchlorid lösung versetzt, bis diese keine Fällung mehr hervorrief. Der dabei entstandene weisse Niederschlag wurde abgesaugt, mit Wasser gewaschen, in Wasser suspendiert und mit Schwefelwasserstoff zerlegt. Das Filtrat vom Quecksilbersulfid lieferte nach dem Verdampfen im Vacuum 11.6 g. salzsaures Histidin (=8.4 g. Histidin). Zur Reinigung wurde es aus wenig Wasser umkrystallisiert und im Vacuum bei 100° getrocknet und analysiert.

0.2120 g Subst.		0.04562 g N	
0.2012 g „		0.0374 g Cl	
		N	Cl
$C_6H_9N_3O_2 \cdot HCl$	Ber.	21.93	18.54
	Gef.	21.52	18.61

Es wurde ferner das Pikrat des Histidins dargestellt, indem man die wässerige Lösung des salzsauren Salzes mit Natrium pikrat versetzte, kurze Zeit erwärmte und erkalten liess. Das Pikrat bestand aus feinen gelben Prismen. Es enthält ein Molekül Krystallwasser, welches bei 50° verloren geht. Die feinen gelben Prismen verwandeln sich dabei zu einer dunkel braunen Krystallmasse.

### C. Der in Wasser unlösliche Rückstand von Bonito fleisch.

Der unlösliche Rückstand von Bonito fleisch wurde getrocknet und fein zerrieben. 100 g. davon wurden mit 700 c.c. 30% iger Schwefelsäure 30 Stunden im Rückflusskühler gekocht. Nach dem Erkalten wurde dieser mit Wasser verdünnt, vom unlöslichen Rückstand abfiltriert und folgende quantitative Analyse ausgeführt.

In 100 Teilen Trockensubstanz.

In Schwefelsäure löslich	92.20
„ unlöslich	7.80
Gesamt stickstoff	14.78
In Schwefelsäure löslicher Stickstoff	14.69
„ unlöslicher Stickstoff	0.09
Ammoniakstickstoff im Schwefelsauren Extrakt	1.02
Durch Phosphowolframsäure fallbarer Stickstoff	4.25
Darunter: { Nucleinbasen stickstoff	Spur
{ Histidin stickstoff	0.71
{ Arginin stickstoff	2.39
{ Lysin stickstoff	1.15
Stickstoff in anderer Form	9.42

Durch Phosphowolframsäure fallbarer Stickstoff als 100.

Nuclein basen stickstoff	Spur
Histidin stickstoff	16.67
Arginin stickstoff	56.18
Lysin stickstoff	27.15

*Isolierung der Hexon basen*

I.—*Histidin*.

Der Schwefelsäure-Extrakt wurde mit fünffacher Menge Wasser verdünnt und mit Phosphorwolframsäure gefällt. Aus dem phosphorwolframsauren Niederschlag wurden die Basen in bekannter Weise freige-  
 macht. Die freie Basen enthaltende, stark alkalische Flüssigkeit wurde mit Kohlensäure gesättigt und mit einer Quecksilberchloridlösung gefällt. Der dabei entstandene weisse Niederschlag wurde in Wasser suspendiert, durch Schwefelwasserstoff zerlegt. Aus dem Filtrate von Quecksilbersulfid schieden sich nach dem Verdampfen farblose, prismatische Krystalle aus. Die Ausbeute derselben betrug ungefähr 1 g. Für die Analyse war das Salz einmal umgelöst, und im Exikator über Schwefelsäure getrocknet.

0.1242 g Subst.		0.02475 g N	
0.1428 g „		0.0235 g Cl	
		N	Cl
$C_6H_9N_3O_2HCl + H_2O$	Ber.	19.93	16.94
	Gef.	19.93	16.46

Das methylestersalzsaure Salz:—Farblose Prismen. Im Kapillarrohr erhitzt schmilzt es bei 197—200° (uncorr) unter lebhaftem Schäumen. Für die Analyse wurde es im Vacuum bei 100° getrocknet.

0.1213 g Subst.		0.0215 g N	
0.1562 g „		0.0458 g Cl	
		N	Cl
$C_6H_8N_3O_2 \cdot (CH_3)_2HCl$	Ber.	17.36	29.34
	Gef.	17.74	29.29

II.—*Arginin*.

Das Filtrat vom Quecksilberchlorid-Niederschlag wurde durch Schwefelwasserstoff vom Quecksilber befreit, im Vacuum verdampft, und mit Silbernitrat versetzt, um die Salzsäure zu entfernen. Zum Filtrat von Chlorsilber wurde Silbernitrat und Baryt im Ueberschuss zugegeben, wobei ein brauner Niederschlag von Arginin silber in reichlicher Menge entstand. Nach dem Zerlegen des Arginin silbers durch Schwefelwasser-

stoff wurde das stark alkalische Filtrat im Vacuum eingedampft und gleich zum Pikrat verwandelt. Die Ausbeute an Pikrat betrug ungefähr 25 g.

Das Pikrat bestand aus gelben Nadeln oder Prismen. Der Schmelzpunkt desselben war  $207-210^{\circ}$  (uncorr).

Für die Analyse war es im Vacuum bei  $100^{\circ}$  getrocknet.

0.2121 g Subst.	0.05116 g N
	N
$C_6H_{14}N_4O_2$ $C_6H_3N_3O_7$	Ber.    24.32
	Gef.    24.12

### III. *Lysin*.

Das Filtrat vom Argininsilber-Niederschlag wurde durch Salzsäure vom Silber und durch Schwefelsäure vom Baryt befreit, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Aus dem phosphowolframsauren Niederschlag wurde 2.5 g. Lysinchlorid gewonnen. Aus dem Chlorid wurde das methylestersalzsaure Salz dargestellt. Es waren farblose Prismen mit dem Schmelzpunkt  $208-210^{\circ}$  (uncorr).

Für die Analyse war es im Vacuum bei  $100^{\circ}$  getrocknet.

0.2050 g Subst.	0.02400 g N
0.2860 g „	0.0382 g Cl
	N                      Cl
$C_7H_{16}N_2O_2 \cdot 2HCl$	Ber.            12.02            30.47
	Gef.            11.71            30.82

### *Zusammenfassung der Resultate*

Aus 1 Kilo getrocknetem Bonito wurden isoliert:

Xanthin	weinig
Hypoxanthin	0.74
Kreatin	Weinig
Histidin	15.00
Carnosin	3.60

Aus 5 Kilo frischem Bonitofleisch wurden isoliert:

Histidin	8.4 g
Kreatin	5.0 g



Aus den Säure spaltungs produkten von 100 g. in Wasser unlöslichen Rückstand des Bonito fleisches wurden isoliert:

Histidin	0.74 g
Arginin	10.8 g
Lysin	2.0 g

## II. LACHS (*Onchorhynchus keta*).

Das frische Lachsfleisch wurde von Haut und Knochen befreit und fein zerhackt. 2900 g. des so zubereiteten Fleisches wurden mit warmem Wasser (40-50°) eine Stunde digeriert und stark abgepresst. Der Rückstand wurde nochmals in derselben Weise behandelt. Diese Operation wurde dreimal wiederholt. Die vereinigten Auszüge wurden mit Bleiessig lösung im kleinem Ueberschuss versetzt. Das Filtrat wurde zuerst durch Schwefelsäure von Blei befreit und mit Schwefelsäure angesäuert bis die Flüssigkeit ungefähr 2% derselben enthielt und so viel Tannin lösung zugegeben, bis diese keinen Niederschlag mehr hervorrief. Nach 24 Stunden wurde es klar abfiltriert und mit einer conc. Lösung von Phosphowolframsäure gefällt. Der dabei entstandene weisse flockige Niederschlag wurde abgesaugt und mit 5% Schwefelsäure gewaschen.

### A. Der Phosphowolframsäure-Niederschlag.

Der Phosphowolframsäure-Niederschlag wurde in gewöhnlicher Weise mit Baryt zerlegt. Die alkalische Flüssigkeit, die freie Basen enthielt, wurde im Vacuum stark eingeeengt und stehen gelassen. Nach einiger Zeit schieden sich farblose glänzende Krystalle aus, was durch Zusatz von Alkohol noch beschleunigt wurde. Die Ausbeute betrug 2.2 g.

Das Rohprodukt wurde aus heissem Wasser umkrystallisiert, im Vacuum bei 100° getrocknet und analysiert.

0.1487 g Subst.	0.2002 g CO <sub>2</sub>	0.0984 g H <sub>2</sub> O		
0.1482 g „	41.c.c. N (18° 764 <sup>mm</sup> )			
		C	H	N
Kreatin C <sub>4</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub> .	Ber.	36.64	6.87	32.06
	Gef.	26.67	7.42	32.13

Die Analyse stimmt also mit dem *Kreatin*. Aus heissem Wasser umkrystallisiert scheidet sich das Kreatin als grosse, durchsichtige, glänzende Prismen oder Stäbchen aus. Bei 100° getrocknet verliert es Krystallwasser und verwandelt sich dabei in ein weisses Pulver. Es bildet kein Doppelsalz mit Zinkchlorid, gibt keine Färbung weder mit wässriger Pikrinsäure und Alkali noch mit Nitoprussidnatrium und Alkali. Die wässrige Lösung des Kreatins reagiert neutral. Im Kapillarrohr erhitzt fängt es von 240° an braun zu werden und zersetzt sich bei höherer Temperatur.

Aus Kreatin wurde leicht Kreatinin dargestellt, indem 1 g. Kreatin in 20 c.c. 10% iger Schwefelsäure gelöst und eine Stunde auf dem Sandbade erhitzt wurde. Nach dem Erkalten wurde die Flüssigkeit durch Baryt von Schwefelsäure befreit und stark eingeeengt, wobei die farblosen Krystalle sich ausschieden, die alle Eigenschaften des Kreatinins hatten. Sie gaben das charakteristische Doppelsalz mit Zinkchlorid, auch rote Färbung mit wässriger Pikrinsäure und Alkali, oder rubinrote Färbung durch Nitoprussid natrium und Alkali.

Die Mutterlauge von Kreatin wurde mit Wasser verdünnt und mit einer wässrigen Quecksilberchloridlösung versetzt. Der weisse flockige Niederschlag wurde durch Schwefelwasserstoff zerlegt, vom Schwefelquecksilber abfiltriert, im Vacuum stark eingeeengt und stehen gelassen. Nach einigen Tagen schieden sich farblose Prismen von salzsaurem *Hypoxanthin* aus, die etwa 1 g. betrugen. Aus heissem Wasser umkrystallisiert verliert es Salzsäure und scheidet sich das Hypoxanthin als mikroskopisch kleine, weisse Krystalle aus, die abgesaugt und mit Wasser gewaschen wurden. Für die Analyse wurde es bei 100° getrocknet.

0.1187 g Subst.		0.1918 g CO <sub>2</sub>		0.0378 g H <sub>2</sub> O
0.1015 g „		36.0 „ N (15° 755 <sup>mm</sup> )		
		C	H	N
C <sub>5</sub> H <sub>4</sub> N <sub>4</sub> O.	Ber.	44.12	2.94	41.18
	Gef.	44.07	3.54	41.26

Die freie Base löst sich ziemlich schwer in kaltem Wasser auf; in Alkohol und Aether ist sie fast unlöslich. Die wässrige Lösung reagiert schwach alkalisch. Der Schmelzpunkt liegt über  $300^{\circ}$ .

Das Hypoxanthin pikrat bestand aus gelben Tafeln, schmolz bei über  $250^{\circ}$ .

In der Mutter lauge von salzsaurem Hypoxanthin war noch eine bedeutende Menge unbekannter Basen vorhanden. Leider war es uns nicht gelungen diese zur Krystallisation zu bringen. Wir haben nur das Vorhandensein von *Histidin* durch Pauly'sche Reaction wahrscheinlich gemacht.

Das Filtrat von Quecksilberchlorid-Niederschlag wurde durch Schwefelwasserstoff von Quecksilber befreit und im Vacuum eingedampft. Nachdem der Schwefelwasserstoff ausgetrieben war, wurde die Salzsäure durch Silbernitrat entfernt; das Filtrat von Chlorsilber wurde nun mit Ueberschuss von Silbernitrat und Baryt versetzt, der dabei entstandene braune Niederschlag wurde mit Schwefelwasserstoff zerlegt. Aus der so erhaltenen alkalischen Flüssigkeit haben wir nichts isolieren können.

Das Filtrat von Silbernitrat und Baryt-Niederschlag wurde durch Salzsäure von Silber und durch Schwefelsäure von Baryt befreit, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Aus dem phosphowolframsauren Niederschlag wurde die Base in bekannter Weise frei gemacht. Die stark alkalische Flüssigkeit wurde im Vacuum eingedampft. Nachdem das Wasser vollständig ausgetrieben war begannen sich farblose Prismen auszuschcheiden und nach einiger Zeit verwandelte sich die ganze Masse in einen Krystallbrei. Sie wurde mit absolutem Alkohol verrieben, abgesaugt und mit Alkohol und Aether gewaschen; die Ausbeute betrug 1.6 g. Zwecks Reinigung wurde die nochmals in wenig Wasser gelöste Masse klar abfiltriert und bei gelinder Wärme langsam verdampft. Die ausgeschiedenen Krystalle wurden abgesaugt, mit Alkohol und Aether gewaschen und für die Analyse im Vacuum bei  $100^{\circ}$  getrocknet.

0.1482 g Subst.	0.2561 g $\text{CO}_2$	0.0986 g $\text{H}_2\text{O}$		
0.1025 g „	20.2 cc N (10° 754 <sup>mm</sup> )			
		C	H	N
$\text{C}_9\text{H}_{14}\text{N}_4\text{O}_3$	Ber.	47.7	6.20	24.77
$\text{C}_9\text{H}_{14}\text{N}_4\text{O}_3 + \frac{1}{4}\text{CO}_2$	Ber.	47.0	6.00	23.63
	Gef.	47.13	7.29	23.71

Die Analyse stimmt am besten mit der Formel  $\text{C}_9\text{H}_{14}\text{N}_4\text{O}_3 + \frac{1}{4}\text{CO}_2$  d.h. *Carnosin* überein; die freie Base scheint Kohlensäure absorbiert zu haben. Wir haben auch einen Grund anzunehmen, dass die freie Base aus der Luft allmählich Kohlensäure absorbiert, denn wir konnten aus dem analysierten Präparat durch unmittelbares Erwärmen mit Pikrinsäure kein Pikrat bekommen. Wenn man aber die Base in Wasser löst, mit Salzsäure schwach ansäuert und kurze Zeit erwärmt, um die Kohlensäure auszutreiben, dann mit einer entsprechenden Menge Natronlauge neutralisiert und mit Pikrinsäure versetzt, so scheidet sich das Pikrat sofort als schöne Prismen aus. Die Kohlensäure dürfte mit der Base ziemlich fest gebunden sein, so dass die Pikrinsäure nicht im Stande ist, sie zu verdrängen. Das freie Carnosin besteht aus langen, farblosen, glänzenden Prismen, leicht löslich in kaltem Wasser, schwer aber in Alkohol. Es hat einen süßen Geschmack, die wässrige Lösung reagiert ziemlich stark alkalisch. In Kapillarrohr erhitzt schmilzt es bei  $230\text{--}232^\circ$  (uncorr.) unter Schäumen.

Carnosin gibt eine weisse Fällung mit Quecksilberchlorid; diese verschwindet aber wenn die letztere Reagens nicht im Ueberschuss vorhanden ist. Es gibt durch Kupfersulfat und Natronlauge eine schwach blau violette Färbung, hingegen keine Färbung durch Pikrinsäure und Alkali. Pauly'sche Reaktion ist auch negativ. Durch Ueberschuss von Silbernitrat und Baryt wird es teilweise gefällt.

*Das Platinchlorid doppelsalz des Carnosins:*—Das Salz wurde dargestellt, indem die freie Base mit verdünnter Salzsäure neutralisiert, mit kleinem Ueberschuss von Platinchlorid lösung versetzt und langsam verdampft wurde. Nach einiger Zeit schieden sich die schönen Prismen

aus, was durch Zusatz von Alkohol beschleunigt wurde. Man krystallisiert das Salz einmal aus Wasser um, und wäscht es mit Alkohol und Aether:

Für die Analyse war es im Vacuum bei 100° getrocknet.

0.0752 g Subst.	5.7 <sup>c.c.</sup> N (16° 758 <sup>mm</sup> )		
0.191 g „	0.0584 g Pt		
		N	Pt
$C_9H_{14}N_4O_3 \cdot 2HCl \cdot PtCl_4$	Ber.	8.81	30.63
	Gef.	8.81	30.58

Das Platin doppelsalz bestand aus schönen Pri-men. Im Kapillarrohr erhitzt fängt es bei 200° braun zu werden an und bei 208° (uncorr.) zersetzt es sich unter Schäumen.

*Carnosin nitrat*:—Zur Darstellung des Nitrats wurde das freie Carnosin mit Salpetersäure neutralisiert und vorsichtig eingedunstet. Es scheidet sich als schöne farblose, meistens stern förmig verwachsene Prismen aus, die in Wasser leicht, in absolutem Alkohol schwer löslich sind; die wässrige Lösung reagiert schwach sauer. Im Kapillarrohr rasch erhitzt zersetzt es sich bei 211°C. (uncorr.) unter Schäumen.

B. *Das Filtrat von Phosphowolframsäure-Niederschlag (A).*

Das Filtrat vom Phosphowolframsäure-Niederschlag A. wurde durch Baryt von Schwefelsäure und Phosphowolframsäure befreit und der Überschuss von Baryt durch Schwefelsäure genau entfernt und im Vacuum bis zum syrup verdampft. Es schieden sich dabei farblose glänzende Krystalle in reichlicher Menge aus, die abgesaugt, mit wenig Wasser, Alkohol und Aether gewaschen wurden. Die Ausbeute betrug 7 g.

Diese Krystalle bestanden fast ausschliesslich aus *Kreatin*. Durch einmaliges Umlösen war es schon vollständig rein. Für die Analyse wurde es im Vacuum bei 100° getrocknet.

0.122 g Subst.	33.9 <sup>c.c.</sup> N (22° 763 <sup>mm</sup> )		
		N	
$C_4H_9N_3O_2$	Ber.	32.06	
	Gef.	31.61	

Die aus Wasser ausgeschiedenen Krystalle sind farblose, glänzende Prismen, ziemlich schwer löslich in Wasser und in Alkohol und Aether fast unlöslich; sie haben kein Drehungsvermögen. Die wässrige Lösung reagiert fast neutral. Mit verdünnter Schwefelsäure erhitzt wurde sie leicht in Kreatinin überführt, was durch Zinkchlorid doppel salz, Pikrinsäure und Alkali und Natriumnitroprussid und Alkali bestätigt wurde.

Die Mutter lauge von Kreatin wurde weiter verdampft, von ausgeschiedenen anorganischen Salzen getrennt, mit absolutem Alkohol versetzt und wieder eingedampft. Der wasserfreie Rückstand wurde jetzt mit absolutem Alkohol übergossen und trockenes Salzsäure gas bis zur Sättigung eingeleitet, sodann wieder eingedampft. Diese Operation wurde nochmals wiederholt. Der nach dem Verdampfen des Alkohols zurückgebliebene dunkel braune Syrup wurde nach der E. Fischer'schen Ester methode nach Monaminosäuren untersucht. Es wurde in der Weise ungefähr 0.3 g. *Alanin* isoliert, welches sich aus der wässrigen Lösung als farblose Prismen ausschied, die den eigentümlichen süßen Geschmack besaßen. In Kapillar rohr erhitzt schmolz es bei  $270^{\circ}$  (uncorr.) unter Zersetzung. Es bildete auch schönes Kupfersalz, das in Wasser leicht löslich war.

Nach Leucin und Glycocoll wurde vergebens gesucht.

*Zusammenfassung der Resultate.*

Aus 1 kilo frischem Lachs fleisch wurden isoliert:

Kreatin	3.2 g
Kreatinin	—
Histidin	Vorhanden
Hypoxanthin	0.28
Carnosin	0.55
Alanin	0.10

### III. MAGURO (*Thynnus thynnus*).

Das frische Maguro fleisch hatte folgende Zusammensetzung.

Wasser	70.68 %
Trocken substanz	29.32 %

In 100 Teilen frischem Fleisch. In 100 Teilen Trockensubstanz

Gesamt stickstoff	4.26	14.86
Gesamt-Extraktivstoffe	10.85	37.00
Stickstoff im Extraktivstoffe	1.93	6.58
Darunter.	<div style="display: flex; align-items: center;"><div style="font-size: 3em; margin-right: 10px;">{</div><div><div>Eiweiss stickstoff</div><div>Durch Phosphowolframsäure</div><div>faellbarer Stickstoff</div><div>Stickstoff in anderer Form</div></div><div><div>0.84</div><div>0.38</div><div>0.71</div><div>2.44</div></div><div><div>2.86</div><div>1.28</div><div></div><div></div></div></div>	

2 Kilo frisches Maguro fleisch, welches vorher von Haut und Knochen befreit und fein zerhackt war, wurde dreimal mit warmem Wasser (40°) extrahiert. Die wässerigen Auszüge wurden mit Tannin und Bleiessig behandelt; das durch Schwefelsäure von überschüssigem Blei befreite Filtrat wurde mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt.

#### A. Der Phosphowolframsäure-Niederschlag.

Die aus dem phosphowolframsauren Niederschlag in bekannter Weise dargestellte, stark alkalische Flüssigkeit, welche freie Basen enthielt, wurde im Vacuum stark eingeeugt. Es schieden sich dabei farblose glänzende Krystalle aus, die ungefähr 2.5 g. betrug. Aus der Mutter lauge wurde durch Zusatz von Alkohol noch 2.5 g. derselben Krystalle erhalten.

Die auf beide Weisen erhaltenen Krystalle wurden aus wenig Wasser umkrystallisiert, bei 100° getrocknet und analysiert.

0.1547 g Subst.	0.2607 CO <sub>2</sub>	0.0952 g H <sub>2</sub> O		
0.1498 g „	35.1 <sup>cc</sup> . N (21°	765 <sup>mm</sup> )		
	C	H	N	
C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>	Ber.	46.45	5.80	27.09
	Gef.	45.95	6.84	26.86

Die Analyse stimmt also mit der Formel C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>, dem *Histidin*, überein. Aus heissem Wasser ungelöst scheidet sich die Base als dünne



perlmutter-glänzende Plättchen aus, welche mit den Leucin krystallen grosse Aehnlichkeit haben. Sie hat fast keinen Geschmack; die wässerige Lösung reagiert schwach alkalisch, sie ist in Wasser leicht, in Alkohol schwer und in Aether nicht löslich. Sie gibt schöne rote Färbung mit alkalischer Diazobenzol sulfosäure Lösung und bildet kein Doppelsalz mit Zinkchlorid, mit dem Millon'schen Reagens gibt sie eine starke weisse Fällung, beim Erwärmen wird sie aber nicht rot. Durch Quecksilberchlorid wird sie als weisser flockiger Niederschlag gefällt.

Die Base gibt auch Biuret reaktion beim Erwärmen, was auch für Histidin charakteristisch ist. Sie hat keinen Schmelzpunkt, im Kapillarrohr erhitzt wird sie bei 240° braun, bei 250° dunkler und zersetzt sich bei höherer Temperatur.

Es wurde das *methylstersalzsaure Salz* dargestellt. Das aus methyl alkoholischer Lösung durch Zusatz von Aethylalkohol und Aether ausgeschiedene Salz wurde im Vacuum bei 100° getrocknet und analysiert.

0.1253 g Subst.	19.9 <sup>cc</sup> . N (20°	757 <sup>mm</sup> )	
0.1050 g „	0.03089 g Cl		
		N	Cl
$C_7H_{11}N_3O_2 \cdot 2HCl$	Ber.	17.36	29.30
	Gef.	17.99	29.41

Im kapillar rohr rasch erhitzt zersetzt sich das Salz bei 197° (uncorr.) unter lebhaftem Schäumen.

*Histidindichlorid.*—Das Salz wurde in gewöhnlicher Weise dargestellt und analysiert.

0.1262 g Subst.	19.9 <sup>cc</sup> . N (20°	762 <sup>mm</sup> )	
0.1595 g „	0.04945 g Cl		
		N	Cl
$C_6H_9N_3O_2 \cdot 2HCl$	Ber.	18.42	31.11
	Gef.	18.09	31.00

Histidin dichlorid scheidet sich aus methylalkoholischer Lösung durch Zusatz von Aethyl alkohol und Aether als farblose Prismen aus. Im Kapillarrohr erhitzt, sintert es bei 232° und zersetzt sich bei 233-234° unter Schäumen.

Wir haben ferner bei unserem Histidin präparate das optische Verhalten untersucht und dasselbe mit dem aus Eiweisskörper durch Hydrolyse erhaltenen Präparate identisch gefunden. viz.

0.324 g freies Histidin in 19.034 g Wasser gelöst, das ein Sp. Gewicht 1.006 hatte, drehte bei 20<sup>cm</sup> Rohr das Natrium licht 1.29° nach links

$$\text{Mithin } [\alpha]_D^{20} = -38.93$$

A. Kossel hat für sein Histidin präparat, das aus den Spaltungsprodukten des Protamins dargestellt wurde, —39.7° angegeben. Der kleine Unterschied ist Beobachtungsfehlern zuzuschreiben.

In salzsaurer Lösung wird die Drehung verändert, wie Kossel auch angegeben hat.

0.2788 g Subst in 21.7152 g 10% iger Salzsäure gelöst, vom Sp. Gw. 1.041 drehte in 20<sup>cm</sup> Rohr das Natrium licht 0.46° nach rechts

$$\text{Mithin } [\alpha]_D^{20} = +17.94$$

Die Mutter lauge von Histidin enthielt noch eine bedeutende Menge Histidin, das von der Mutter lauge schwer zu trennen war; zu diesem Zwecke wurde sie in 500 c.c. Wasser gelöst und mit einer conc. wässerigen Lösung von Quecksilberchlorid versetzt. Der weisse flockige Niederschlag wurde abgesaugt, mit wenig Wasser gewaschen, in Wasser verteilt und durch Schwefelwasserstoff zerlegt. Das Filtrat von Schwefel quecksilber wurde im Vacuum eingedampft und in bekannter Weise das methyl ester salzsaure Salz des Histidins dargestellt. Die Ausbeute betrug ungefähr 6.8 g. (entsprechend 4.4 g. freies Histidin).

Aus 2 kilo frischem Fleisch haben wir somit im ganzen 9.4 g. freies Histidin isoliert.

Aus der Analyse und anderen Beobachtungen wurde das Salz mit dem vorher von Bonito gewonnenen Präparat vollständig identisch gefunden.

Das Filtrat vom Quecksilberchlorid-Niederschlag wurde durch Schwefel wasserstoff vom Quecksilber befreit und nach dem Entfernen der Salzsäure durch Silbernitrat wurde es mit Ueberschuss von Silbernitrat

und Baryt versetzt. Aus dem dabei entstandenen braunen Niederschlag konnte man jedoch keine Base in genügender Menge isolieren.

Das Filtrat von Silber nitrat und Baryt-Niederschlag wurde nach dem Entfernen des Silbers durch Salzsäure und des Baryts durch Schwefelsäure, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Die aus dem phosphowolframsauren Niederschlag in bekannter Weise dargestellte alkalische Flüssigkeit wurde gleich mit Pikrinsäure versetzt. Das in dieser Weise dargestellte Pikrat betrug ungefähr 8 g.

Aus wenig heissem Wasser umgelöst scheidet sich das Pikrat als Aggregat von mikroskopisch feinen citronen gelben Nadeln aus. Im Kapillar rohr erhitzt wird es von 200° an allmählich braun und zersetzt sich bei 216° (uncorr.)

Für die Analyse wurde es im Vacuum bei 100° getrocknet.

0.1418 g Subst.	0.2047 g CO <sub>2</sub>	0.0605 g H <sub>2</sub> O			
0.1471 g „	26.9 <sup>cc</sup> . N (18°	767 <sup>mm</sup> )			
0.4836 g „	0.2454 g Pikrinsäure				
	C	H	N	Pikrinsäure	
C <sub>9</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> · C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>	Ber.	39.56	3.74	21.54	50.33
	Gef.	39.88	4.47	21.29	50.74

Aus dem Pikrate wurde das Nitrat der Base dargestellt; zu diesem Zwecke wurden 2 g. Pikrat in wenig heissem Wasser gelöst, mit Ueberschuss von Salzsäure versetzt, von der dabei ausgeschiedenen Pikrinsäure abfiltriert, und wiederholt mit Aether geschüttelt, um die darin vorhandene Pikrinsäure vollständig zu entfernen und dann mit Phosphowolframsäure gefällt. Der Phosphowolframsäure-Niederschlag wurde in bekannter Weise durch Baryt zerlegt. Die alkalische Flüssigkeit wurde mit Salpetersäure neutralisiert und bis zum Syrup eingengt. Die ganze Masse verwandelte sich bald in farblose Prismen, die sich meistens sternförmig zusammengruppierten. Diese wurden mit wenig absolutem Alkohol verrieben, abgesaugt und nochmals aus wenig Wasser umgelöst. Für die Analyse wurden sie im Vacuum bei 100° getrocknet.

0.116 g Subst.	23.6 <sup>cc</sup> N (11° 764 <sup>mm</sup> )		
0.3845 g „	0.492 g Nitron nitrat (nach Nitron methode)		
		N	HNO <sub>3</sub>
C <sub>9</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> · HNO <sub>3</sub>	Ber.	24.22	21.80
	Gef.	24.40	21.50

Das Nitrat bestand aus farblosen Prismen; es ist in Wasser sehr leicht, in Alkohol schwer und in Aether unlöslich. Im Kapillarrohr erhitzt schmilzt es bei 211° (uncorr.) zu einem Oel und zersetzt sich gleich darauf.

Aus dem Pikrate wurde die freie Base dargestellt. Sie bestand aus farblosen Prismen, reagierte zieml stark alkalisch und zersetzte sich bei 230° (uncorr.) unter Schäumen.

Alle diese Eigenschaften stimmen mit dem *Carnosin* aus Lachs überein.

Wir haben auch das optische Verhalten des Nitrates untersucht:

0.4881 g Carnosin nitrat in 15.014 g Wasser gelöst, bei eniem Spezifischen Gewicht von 1.008, drehte im 20<sup>cm</sup> Rohr Das Natriumlicht 0.74° nach rechts

$$\text{Mithin} \quad \alpha_D^{20} = +11.66$$

Vergleicht man nun diese Zahl mit jener des Carnosin nitrates aus Liebig'schem Fleischextrakt  $\alpha_D^{20} = +22.3$  so findet man, dass sie beinahe die Hälfte der letzteren ist. Der Schmelzpunkt der freien Base liegt auch einige grade niedriger. Wahrscheinlich handelt es sich um eine Isomerie, was später noch eingehender untersucht werden soll.

### B. Das Filtrat von phosphowolframsaurem Niederschlag.

Aus dem Filtrate von phosphowolframsaurem Niederschlag wurde 6 g. reines *Kreatin* isoliert und in der Mutterlauge von Kreatin das Vorhandensein kleiner Mengen *Monamino*säuren mittelst Ester methode nachgewiesen; zur Analyse genügte die Menge jedoch nicht.

### Zusammenfassung der Resultate.

Aus 2 kilo frischem Fleisch wurde isoliert.

Kreatin	6.0
Kreatinin	—
Histidin	94
Hypoxanthin	—
Carnosin	40
Alanin	Vorhanden

#### IV. HUMMER (*Isc-Yebi*: *Panulirus. sp.*)

##### A. Das Hummer fleisch.

33 Stücke frische Hummern (3710 g.) wurden von den Schalen abgelöst und das daraus gesammelte Fleisch, das frisch gewogen 1712 g. betrug, wurde fein zerhackt, mit warmem Wasser (60—70°) dreimal extrahiert. Mit dem Extrakte wurde zuerst eine quantitative Analyse angeführt.

##### In 100 Teilen frischem Fleisch

In Wasser löslicher Stickstoff	2.78
Darunter: { Eiweiss Stickstoff	0.71
{ Durch Phosphowolframsäure fällbarer	
Stickstoff	1.23
{ Stickstoff in anderer Form	0.84

##### In Wasser löslicher Stickstoff als 100

Eiweiss stickstoff	25.5
Durch Phosphowolframsäure fällbarer	
Stickstoff	44.3
Stickstoff in anderer Form	30.2

Der Rest des Extraktes wurde mit Essigsäure angesäuert und mit Tannin gefällt; der dabei entstandene flockige Niederschlag wurde abgesaugt und das Filtrat davon wurde mit Bleiessig versetzt. Das Filtrat vom Blei-Niederschlag wurde nach dem Entfernen des Bleies durch Schwefelsäure, mit so viel Schwefelsäure angesäuert, bis die Flüssigkeit ungefähr 5% derselben enthielt und mit Phosphowolframsäure gefällt.



		$\text{H}_2\text{O}$	
$\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$	$\text{C}_6\text{H}_3\text{N}_3\text{O}_7 + 2\text{H}_2\text{O}$	Ber.	8.20
		Gef. 1)	8.19
		2)	8.13

Um das Pikrat in *salzsauren Methylester des Arginins* zu verwandeln wurde 3 g. Pikrat in wenig heissem Wasser gelöst, 20 c.c. verdünnter Salzsäure (1:3) zugegeben, von der ausgeschiedenen Pikrinsäure getrennt und wiederholt mit Aether geschüttelt, bis die Flüssigkeit ganz farblos wurde. Die wässrige Lösung wurde dann im Vacuum verdampft und in gewöhnlicher Weise durch Methyl alkohol und Salzsäure in das methylester salzsaure Salz verwandelt. Zur Reinigung wurde das Salz in wenig heissem Methyl alkohol gelöst, mit Tierkohle entfärbt und durch Zusatz von Aethyl alkohol und Aether ausgeschieden. Für die Analyse wurde es im Vacuum bei  $80^\circ$  getrocknet.

0.157 g Subst.	0.0424 g AgCl		
0.1511 g „	27.6 c.c. N ( $12^\circ$	756 <sup>mm</sup> )	
	N	Cl	
$\text{C}_7\text{H}_{16}\text{N}_4\text{O}_2\text{HCl}$	Ber.	21.47	27.15
	Gef.	21.63	27.02

Das salzsaure Salz bestand aus farblosen Prismen, die sich meistens sternförmig aneinander gruppieren. Es löst sich in Wasser und Methylalkohol leicht, in Aethylalkohol etwas schwerer; in Aether, Petrolether und Chloroform ist es fast unlöslich. Im Kapillarrohr rasch erhitzt zersetzt es sich bei  $183^\circ$  (uncorr.) unter lebhaften Schäumen.

Das optische Verhalten des isolierten Arginins soll später untersucht werden.

Die zweite Fraktion war das Gemisch von Arginin und Lysin pikrat. Die Ausbeute derselben betrug 11.7 g.

Aus der dritten Fraktion wurde 1.5 g. *Lysin pikrat* in reinem Zustande gewonnen. Es bestand aus hellgelben langen Prismen, enthält kein Krystallwasser. In Wasser und Alkohol war es ziemlich leicht, in Aether aber schwer löslich. Im Kapillarrohr erhitzt wurde es bei  $230^\circ$  braun und zersetzte sich bei höherer Temperatur.



Für die Analyse war es im Vacuum bei 100° getrocknet.

0.1698 g Subst.	0.2392 g CO <sub>2</sub>	0.0695 g H <sub>2</sub> O	
0.1256 g „	19.9 <sup>c.c.</sup> N (15.5°	757 <sup>mm</sup> )	
0.5078 g „	0.3098 g Pikrinsäure		
	C	H	N
C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> · C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>	Ber. 38.40	4.53	18.67
	Gef. 38.38	4.55	18.44
			61.07
			61.01

Aus dem Pikrate wurde das methyl ester salzsaure Salz in bekannter Weise<sup>1</sup> dargestellt. Es war farblose Prismen mit dem Schmelzpunkt 216°—218°. Für die Analyse war es im Vacuum bei 100° getrocknet.

0.1495 g Subst.	15.0 <sup>c.c.</sup> N (12°	769 <sup>mm</sup> )	
0.1680 g „	0.05025 g Cl		
	N	Cl	
C <sub>7</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub> · 2HCl	Ber. 12.07	30.47	
	Gef. 12.05	29.91	

2. Die zweite Portion wurde mit Kohlensäure gesättigt und mit einer wässrigen Quecksilberchlorid lösung versetzt. Aus dem Quecksilberchlorid-Niederschlag wurde 2 g. *Argininmethylester salzsauresalz* isoliert.

Das Vorhandensein von *Histidin* wurde durch Diazobenzol sulfosäure nachgewiesen; gelang es uns jedoch nicht, diese Base in reinem Zustande zu isolieren.

Das Filtrat vom Quecksilberchlorid Niederschlag wurde nach dem Entfernen des Quecksilbers durch Schwefelwasserstoff und der Salzsäure durch Silber nitrat, mit Silber nitrat und Baryt in kleinem Ueberschuss versetzt. Aus dem dabei entstandenen braunen Niederschlag wurde 4 g. Arginin pikrat gewonnen.

Das Filtrat vom Silbernitrat und Baryt Niederschlag lieferte noch 1.4 g. reines Lysin pikrat.

h). *Das Filtrat von Phosphowolframsäure-Niederschlag.*

Das Filtrat von phosphowolframsäurem Niederschlag (a) wurde

1. Vergl.: E. Fischer u. U. Suzuki: Berichte d. deutsch. chem. Gesellschaft, XXXVIII, Band III, 4180.

nach dem Entfernen der Schwefelsäure und Phosphowolframsäure durch Baryt und des Ueberschlusses von Baryt durch Schwefelsäure, im Vacuum stark eingengt. Es schieden sich dabei Tyrosin und Leucin aus; sie wurden in 4 Fraktionen gesammelt und zwar.

I	Fraktion	0.95 g	bestand hauptsächlich aus Tyrosin
II	„	0.92 g	
III	„	0.90 g	Gemisch von Tyrosin und Leucin
IV	„	1.45 g	Leucin

1. *Tyrosin*:—Aus der I Fraktion wurde das Tyrosin rein dargestellt, bei 100° getrocknet und analysiert.

0.1308 g Subst.	0.2831 g CO <sub>2</sub>	0.0728 g H <sub>2</sub> O		
0.1182 g „	7.9 <sup>cc.</sup> N (16°	751 <sup>mm</sup> )		
	C	H	N	
C <sub>9</sub> H <sub>11</sub> NO <sub>3</sub>	Ber.	59.67	6.07	7.73
	Gef.	59.03	6.18	7.69

Aus heissem Wasser unkrystallisiert, scheidet sich das Tyrosin aus; seidenglänzende Nadeln, schwer löslich in Wasser, fast unlöslich in Alkohol und Aether. Es gibt schöne rote Färbung mit Millon'schen Reagens, auch rote Färbung mit Diazobenzolsulfosäure in alkalischer Lösung.

2. *Leucin*:—Aus der IV Fraktion; durch zweimalige Umkrystallisation war es gelungen das Leucin in reinem Zustande zu isolieren. Für die Analyse wurde es bei 100° getrocknet.

0.1500 g Subst.	0.2921 g CO <sub>2</sub>	0.1343 g H <sub>2</sub> O		
0.1433 g „	13.5 <sup>cc.</sup> N (15°	753 <sup>mm</sup> )		
	C	H	N	
C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>	Ber.	54.96	9.92	10.69
	Gef.	53.11	9.94	10.93

Aus heissem Wasser scheidet sich das Leucin als dünne, perlmutterglänzende Plättchen aus; es enthält kein Krystallwasser, hat kaum Geschmack. Es löst sich in Wasser, ist aber in Alkohol und Aether fast unlöslich. Die wässrige Lösung reagiert fast neutral. Es bildet schönes Kupfersalz, das in Wasser schwer löslich ist.

Aus der Mutter lauge von Tyrosin und Leucin wurde durch Easter Methode noch 2 g. reines *Leucin*, ungefähr 1 g. *Alanin* und kleine Menge *Prolin* gewonnen. Wegen Mangel an Zeit haben wir jedoch die beiden letzteren Körper nicht analysiert.

## B. Die Hummer schale

33 Stücke Hummern (3710 g.) lieferte 1415 g. frische Schale, die vollständig vom Fleisch befreit war. Diese wurde fein zerrieben, mit heissem Wasser extrahiert und genau so bearbeitet wie das Fleisch.

### In 100 Teilen frischer Schalen

In Wasser löslicher Stickstoff	1.12
Darunter: { Eiweiss stickstoff	0.32
{ Durch Phosphowolframsäure fällbarer	
{ Stickstoff	0.49
{ Stickstoff in anderer Form	0.31

### In Wasser löslicher Stickstoff als 100

{ Eiweiss stickstoff	28.6
{ Durch Phosphowolframsäure fällbarer	
{ Stickstoff	43.7
{ Stickstoff in anderer Form	27.7

Aus dem phosphowolframsauren Niederschlag wurde 1.5 g. *Lysin pikrat* in reinem Zustande isoliert.

0.1531 g Subst.	0.2142 g CO <sub>2</sub>	0.064 g H <sub>2</sub> O
0.1524 g „	23.8 <sup>c.c.</sup> N (13°	768 <sup>mm</sup> )
0.7033 g „	0.430 g Pikrinsäure	

		C	H	N	Pikrinsäure
C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> · C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub> ·	Ber.	38.40	4.53	18.67	61.07
	Gef.	38.16	4.65	18.65	61.14

Ausserdem haben wir noch eine Base als Pikrat isoliert, die Ausbeute desselben betrug ungefähr 4 g. Diese Base soll später näher untersucht werden.

Wir haben ferner das Vorhandensein von *Tyrosin*, *Leucin*, *Alanin*, *Prolin* (?) und *Histidin* nachgewiesen. Zur Analyse reichte die Menge jedoch nicht aus.

*Zusammenfassung der Resultate.*

Aus 1 Kilo frischem Fleisch und frischer Schale wurden isoliert.

	Hummer fleisch	Schale
Arginin	3.3	—
Lysin	0.66	0.42
Histidin	vorhanden	vorhanden
Leucin	2.3	„
Tyrosin	1.36	„
Alanin	0.6	„
Prolin	vorhanden	„

V. SURUME-IKA (*Ommastrephes sp.*)

Der getrocknete Surume-Ika hat folgende quantitative Zusammensetzung.

In 100 Teilen Luft trockensubstanz.

Wasser	23.09
Trockensubstanz	76.91

In 100 Teilen Trockensubstanz

Organische substanz	92.25
Asche	7.75
Gesamt Phosphor	2.85
In heissem Wasser löslicher Phosphor	2.01
Gesamt Stickstoff	14.96
In heissem Wasser löslicher Stickstoff	4.90

Darunter:	{ Ammoniak stickstoff	0.27
	{ Eiweiss stickstoff	2.45
	{ Nicht-Eiweiss stickstoff	2.19
	{ Durch Phosphowolframsäure fällbarer Stickstoff	1.50

In heissem Wasser löslicher Stickstoff als 100

Ammoniak stickstoff	5.43
Eiweiss stickstoff	49.98
Nicht-Eiweiss stickstoff	44.59
Durch Phosphowolframsäure fällbarer Stickstoff	30.58
Stickstoff in anderer Form	10.01

A). *Taurin* und *Leucin*.

500 g. Luft trocken substanz wurden mit warmem Wasser (50°C.) wiederholt extrahiert. Die wässrigen Auszüge wurden nach dem Behandeln mit Tannin und Bleiessig lösung in bekannter Weise im Vacuum stark eingeeengt und im Exikator stehen gelassen. Nach mehreren Tagen schieden sich die grossen monoklinischen Prismen aus, die ungefähr 3.7 g. betrugen. Diese Krystalle wurden aus heissem Wasser umkrystallisiert, bei 100° getrocknet und analysiert.

0.3252 g Subst. gab	0.0365 g N		
0.1540 g „	0.2874 g BaSO <sub>4</sub>		
	N	S	
C <sub>2</sub> H <sub>7</sub> NSO <sub>3</sub> (Taurin)	Ber.	11.20	25.60
	Gef.	11.23	25.63

Aus der Mutter lauge wurden durch Zusatz von Alkohol noch 4.3 g. Krystalle gewonnen, die unter dem Mikroskop betrachtet aus zwei verschiedenen Körpern bestanden. Der eine war Taurin und der andere Leucin. Durch Fraktionieren wurde zuerst 3 g Taurin in reinem Zustande gewonnen.

0.1667 g Subst.		0.01865 g N	
0.1780 g „		0.04611 g S	
		N	S
$C_2H_7NSO_3$	Ber.	11.20	25.60
	Gef.	11.19	25.79

Im ganzen haben wir somit 6.7 g. Taurin isoliert.

Die von Taurin getrennte Mutterlauge lieferte 0.5 g. *Leucin*, das unmittelbar in das Kupfersalz verwandelt und analysiert wurde.

0.1700 g Subst.		0.0332 g Cu	
		Cu	
$(C_6H_{12}NO_2)_2Cu$	Ber.	19.62	
	Gef.	19.53	

#### B). Organische Basen.

Es wurde zuerst die quantitative Analyse ausgeführt.

In 100 Teilen Stickstoff der organischen Basen:

Durch Silbernitrat in neutraler Reaktion fällbarer Stickstoff.	4.27
Durch Silber nitrat und Baryt fällbarer Stickstoff	31.71
Stickstoff in anderer Form	64.02

500 g. lufttrockenes Material wurde mit heissem Wasser extrahiert. Nach dem Proteinstoffe und andere Verunreinigungen durch Tannin und Bleiessig lösung beseitigt waren, wurde die Flüssigkeit mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Aus dem phosphowolframsauren Niederschlag wurde in bekannter Weise eine stark alkalische Flüssigkeit der freien Base gewonnen. Diese Flüssigkeit wurde jetzt mit Salpetersäure neutralisiert und mit Silbernitrat versetzt. Der dabei entstandene Niederschlag war in verhältnismässig sehr geringer Menge, in diesem Niederschlag wurde nur das Vorhandensein von *Hypoxanthin* und *Xanthin* identifiziert.

Das Filtrat von Silbernitrat-Niederschlag lieferte durch Zusatz von Silber nitrat und Baryt im Ueberschuss einen braunen Niederschlag,

aus dem ungefähr 1.5 g. salzsaures Salz einer unbekannten Base, deren Natur nicht näher aufgeklärt werden konnte, dargestellt wurde.

Das Filtrat von Silbernitrat und Baryt-Niederschlag wurde nach dem Entfernen des Silbers durch Salzsäure und des Baryts durch Schwefelsäure, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Aus dem phosphowolframsauren Niederschlag wurde die Base wieder frei gemacht. Die stark alkalische Flüssigkeit lieferte nach dem Einengen und längeren Aufbewahren im Exikator grosse farblose monoklinische Krystalle, die ungefähr 8 g. betrugen.

Diese Krystalle wurden aus heissem Alkohol umkrystallisiert. Für die Analyse wurden sie im Vacuum bei 80° getrocknet.

1.	0.1498 g	Subst.	0.2825 g	CO <sub>2</sub>	0.1327 g	H <sub>2</sub> O
2.	0.1561 g	„	0.2928 g	CO <sub>2</sub>	0.1325 g	H <sub>2</sub> O
3.	0.1491 g	„	15.5 <sup>c.c.</sup>	N (13 <sup>0</sup> .	757 <sup>mm</sup> )	
4.	0.1524 g	„	15.7 <sup>c.c.</sup>	N (14.5 <sup>0</sup>	763 <sup>mm</sup> )	
			C		H	N
C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	Ber.		51.28		9.40	11.97
	Gef.	1.	51.42		9.84	12.24
		2.	51.16		9.43	12.14

Die Base hat einen angenehmen süssen Geschmack; löst sich leicht in Wasser und in heissem Alkohol.

Sie enthält ein Molekül Krystallwasser, das im Vacuum bei 100° verloren geht.

0.4230 g Subst. (im Exikator über Schwefelsäure getrocknet)  
 verlor im Vacuum bei 100° 0.0509 g Wasser

C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub> + H <sub>2</sub> O	Ber.	13.33
	Gef.	12.03

Die Analyse stimmt also mit der Formel C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub>, der *Aminovaleriansäure* überein.

Das Platinchlorid doppelsalz bestand aus gold gelben monoklinischen Tafeln, die sich entweder aus Wasser oder aus Alkohol umkrystallisieren liessen. Für die Analyse wurden sie bei 100° getrocknet.



0.1700 g Subst (Aus Wasser umkryst.) gab	0.05120 g Pt
0.1358 g „ (Aus Alkohol umkryst) „	0.0410 g Pt
	Pt
$(C_5H_{11}NO_2 \cdot HCl)_2PtCl_4$	Ber. 30.29
	Gef. 1. } 30.12
	2. } 30.19

Im Kapillar rohr erhitzt zersetzt sich das Platindoppelsalz bei 246-247° (uncorr.).

Die Base bildet auch das Pikrat. Es bestand aus gelben Prismen, die in heissem Wasser leicht, in kaltem Wasser aber schwer löslich waren. Im Kapillarrohr erhitzt zersetzt es sich bei 235° (uncorr.).

Von den, oben erwähnten Beobachtungen und besonders von der basischen Eigenschaften der Base und der Fällbarkeit durch Phosphowolframsäure halten wir diese Base für *δ-Aminovaleriansäure*, die von Salkowski aus gefaulten Pankreas isoliert und später von Ackerman näher untersucht worden ist. Genauere Studien der Base hoffen wir bald mittheilen zu können.

## VI. UNAGI (*Suesswasser Aal: Anguilla fluviatilis*).

Das frische Fleisch von Aal enthielt:

Wasser	69.24
Trockensubstanz	30.76

In 100 Theilen Trockensubstanz.

Fett	37.48
Gesamt stickstoff	9.58
Eiweiss stickstoff	8.79
Nicht-Eiweiss stickstoff	0.79
Ammoniak stickstoff	Spur.
In Wasser löslicher Stickstoff	3.62
Darunter:	
Eiweiss stickstoff	2.83

Ammoniak stickstoff	Spur.
Nicht-Eiweiss stickstoff	0.79
Durch Phosphowolframsäure fällbarer	
Stickstoff	0.30
Stickstoff in anderer Form	0.49

#### A. Organische Basen.

13 Kilo frisches Fleisch wurde mit warmem Wasser extrahiert und in gewöhnlicher Weise die Basen durch Phosphowolframsäure gefällt. Die aus dem phosphowolframsauren Niederschlag dargestellten freien Basen wurden durch Silbernitrat und Baryt gefällt. Von diesem Niederschlag wurde 8.6 g. freies *Carnosin* gewonnen. Dies wurde aus wenig Wasser umgelöst, im Vacuum bei 100° getrocknet und analysiert.

0.1252 g Subst.	0.2180 g CO <sub>2</sub>	0.0701 g H <sub>2</sub> O		
0.1608 g Subst.	31.8 <sup>cc.</sup> N (0°, 760 <sup>mm</sup> )			
	C	H	N	
C <sub>9</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub>	Ber.	47.70	6.20	24.77
	Gef.	47.50	6.22	24.81

Das freie Carnosin bestand aus farblosen Nadeln oder Prismen; in Wasser löst es sich leicht, in Alkohol aber schwer. Die wässrige Lösung reagiert stark alkalisch.

Das Kupfersalz:—Dunkelblaue, sechseckige Tafeln. In Kapillarrohr erhitzt zersetzt es sich bei 220° ohne zu schmelzen. Es ist in heissem Wasser leicht, in kaltem Wasser aber schwer löslich. Für die Analyse wurde es bei 100° getrocknet.

0.1936 g Subst.	0.0412 g Cu		
	Cu		
C <sub>9</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> · CuO	Ber.	20.81	
	Gef.	21.29	

Das Platinchlorid doppelsalz:—Gelbe Prismen, löslich in Wasser und Alkohol; unlöslich in Aether. Der Schmelzpunkt liegt bei 210—222°.

Für die Analyse war das Salz bei 100° getrocknet.

0.1726 g Subst.	0.0519 g Pt
	Pt
$C_9H_{14}N_4O_3 \cdot 2HCl. PtCl_4$	Ber. 30.61
	Gef. 30.06

Das Nitrat:—Farblose Prismen; Schmelzpunkt  $211^\circ$

0.1174 g Subst. gab	0.02478 g $HNO_3$
	$HNO_3$
$C_9H_{14}N_4O_3 \cdot HNO_3$	Ber. 21.80
	Gef. 21.11

B. Das Filtrat vom phosphowolframsauren Niederschlag.

Aus dem Filtrat vom phosphowolframsauren Niederschlag wurden 8.4 g. Kreatin isoliert. Mit dem gereinigten Präparate wurde Krystall wasser-und Stickstoff bestimmung ausgeführt.

0.1498 g Subst Verlor bei $100^\circ$	0.0184 g Wasser
	Wasser
$C_4H_9N_3O_2 + H_2O$	Ber. 12.12
	Gef. 12.28
0.1314 g Subst (bei $100^\circ$ getrocknet)	0.0426 g N
	N
$C_4H_9N_3O_2$	Ber. 32.06
	Gef. 32.42

Das Kreatin bestand aus farblosen Prismen, welche bei  $100^\circ$  ihr Krystallwasser verlieren und in ein undurchsichtiges, weisses Pulver sich verwandeln. Im Kapillar rohr erhitzt wird es bei  $255^\circ$  schwarz braun und zersetzt sich bei höherer Temperatur.

*Zusammenfassung der Resultate.*

Aus 13 Kilo frischem Fleisch wurden isoliert.

Kreatin	8.4 g
Carnosin	8.6 g

Die Schleimige Substanz von Aal besteht wahrscheinlich aus einem Mucinähnlichen Körper. In ziemlich reinem Zustande enthielt sie 11.83% N.

## TABELLE DER EXTRAKTIV STOFFE IM FISCH FLEISCHE.

(Auf 1 kilo frischem, bezw. getrocknetem Material berechnet).

	Bonito		Maguro	Lachs		Hummer		Ika	Aal
	Getrockneter Bonito	Frisches Fleisch	Frisches Fleisch	Frisches Fleisch	Frisches Fleisch	Frisches Fleisch	Frische Schale	Getrocknetes Fleisch	Frisches Fleisch
Arginin	1 kilo	1 kilo	1 kilo	1 kilo	1 kilo	1 kilo	1 kilo	1 kilo	1 kilo
Lysin	—	—	—	—	—	{3.3 2.85(*) 0.66	—	—	—
Histidin	—	—	—	—	—	0.42	—	—	—
Naudin	15.0 g	1.7	4.7	Vorhanden	Vorhanden	Vorhanden	Vorhanden	—	—
Hypoxanthin	0.74	—	—	0.28	—	—	—	—	—
Carnosin	3.60	1.0	2.0	0.55	—	—	—	—	0.67
Kreatin	Vorhanden	—	3.0	3.2	—	—	—	—	0.65
Kreatinin	—	—	—	—	—	—	—	—	—
Taurin	—	—	—	—	—	—	—	17.4	—
Leucin	—	—	—	—	—	2.3	Vorhanden	1.3	—
Tyrosin	—	—	—	—	—	1.36	Vorhanden	—	—
Alanin	—	—	Vorhanden	0.10	—	0.60	Vorhanden	—	—
$\beta$ -Aminovaleriansäure (?)	—	—	—	—	—	—	—	20.8	—
Prolin	—	—	—	—	—	Vorhanden	Vorhanden	—	—

\* Gemisch von Arginin. u. Lysin.

Zum Schluss möchten wir Herrn Assistent A. Ōtake für seine eifrige Hilfe bei dieser Arbeit unseren besten Dank aussprechen.

**Hydrolyse der wilden Seiden<sup>1</sup>; Antheraea Peryni Guér  
(Sakusan), Antheraea Yamamai Guér (Yamamai)  
und Caligula Japonica Moore (Kuriwata.)**

VON

**U. Suzuki, K. Yoshimura und R. Inouye.**

Die gewöhnliche Seide von *Bombyx mori* ist, wegen ihrer technischen Wichtigkeit und ihres physiologischen Interesses mehrfach von verschiedenen Seiten untersucht worden. Die als Handelsartikel minder wertigeren, trotzdem immer noch eine wichtige Rolle spielenden wilden Seidenarten, wie *Antheraea Peryni* (Sakusan), *Antheraea Yamamai* (Yamamai) und *Caligula Japonica* (Kuriwata) sind dagegen viel seltener erforscht; besonders fehlt es an gründlichen Studien über die chemische Zusammensetzung derselben.

Die gewöhnliche Seide lässt sich durch Einwirkung von Seifen, Alkalien oder sogar von heissem Wasser in zwei verschiedene Bestandteilen trennen, nämlich das Fibroin und das Sericin. Das erstere bildet den Hauptanteil der Seide und ist gegen die obengenannten Reagenzien viel beständiger als das letztere.

Die Hydrolyse des Fibroins ist schon von verschiedenen Autoren unternommen worden. So haben Hinterberger<sup>2</sup> und Watenburger<sup>3</sup> in den Spaltungsprodukten des Fibroins durch Salzsäure, Tyrosin isoliert; sie wollten auch das Leucin gefunden haben. Staedler<sup>4</sup> und Cramer<sup>5</sup> gelangten auch zu denselben Resultaten; letzterer fand ausserdem noch Glykokoll. Schützenberger<sup>6</sup> und Burgeois haben das Fibroin mit Baryt-

1. Diese Abhandlung ist schon in "the Journal of the Tokyo Chemical Society." Bd. 28 heft 11. (Nov. 1907) publiziert worden

2. Hinterberger: Jahres. f. Chem. 1853. 615

3. Watenburger: Wien. Akad. Ber. 11 450

4. Staedler: Ann. 1859 111 12

5. Cramer: sour. f. prakt. Chem. 1865 96 76

6. Schützenberger: Comptes rendus 1875. 31 1191

wasser unter Druck erhitzt und behaupteten, neben Ammoniak, Oxalsäure, Kohlensäure und Essigsäure ein Gemisch von Aminosäuren, das aus Tyrosin, Glykokoll, Alanin, Aminobuttersäure und einer ungesättigten Säure  $C_4H_7NO_2$  bestehen sollte, gefunden zu haben. Ihre Angabe ist unzuverlässig, weil sie keine genauere Beschreibung der Methode gegeben haben. Ferner ist der Baryt kein geeignetes Mittel für Hydrolyse, weil er bei höherer Temperatur sekundäre Zersetzungen bewirken kann.

Weyl<sup>6</sup> hat wieder die Hydrolyse durch Säure ausgeführt und neben Tyrosin und Glykokoll, noch eine Aminopropionsäure gefunden, die er für d-Alanin hielt. Diese drei Körper hat er in reinem Zustande isoliert; es gelang ihm jedoch nicht Lencin nachzuweisen.

Über die Diaminosäuren liegen einige Angaben von Wetzel<sup>7</sup> vor.

Ein grosser Fortschritt auf diesem Gebiete ist aber E. Fischer<sup>8</sup> und seinem Mitarbeiter A. Skita zu verdanken. Diese Autoren haben die bekannte Ester methode, die für die Forschung der Spaltungsprodukten der Eiweisskörper so grosse Erfolge gebracht hat, für Seiden Fibrin und Sericin angewendet, und ausser den bisher bekannten Stoffen: Glykokoll, Alanin und Tyrosin, nicht nur das Vorhandensein von Serin, Phenylalanin, Prolin, Leucin. Arginin etc nachgewiesen sondern auch das optische Verhalten dieser Körper festgestellt. Unentschieden bleibt noch die Existenz von Lysin, und Valin im Fibrin. In Sericin ist auch nach Lysin und Histidin zu suchen.

Es sei noch erwähnt, dass E. Fischer<sup>9</sup> später auch Spinnen seide aus Madagasear "*Nephilia madagascariensis*" (*Soie d'araignée de Madagascar*) untersucht hat. Seine Angaben haben wir zum Vergleich mit unserem Resultate, in einer Tabelle zusammengestellt.

Der Hauptzweck unserer Arbeit ist die Spaltungs produkte der wilden

6. Weyl: Ber. 1888. 21 1407 u. 1529

7. Wetzel: Zeitsch. f. Physiol. Chem. 1899 26 535

8. F. Fischer u. A. Skita: Zeitsch. f. Physiol. Chem. (1901) 33 177 1902 35 221. (1903) 39 155

9. F. Fischer: Ueber Spinnenseide: Sitzungsberichte. Kgl. pr. Akad. Wiss. Berlin (1907) 440-50

Seiden möglichst genau kennen zu lernen und mit denjenigen der gewöhnlichen Seide zu vergleichen.

Wir geben nur zu, dass unsere Ergebnisse noch viele Lücken haben, die wir später noch auszufüllen hoffen.

### I.—Sakusan-Seide. (*Antheraea Pernyi* Guér.)

Die käufliche Sakusan-Seide, die wir zu dieser Untersuchung anwendeten, hatte folgende Zusammensetzung.

Wasser	13.16 %
Trockensubstanz	86.84 %

#### In 100 Teilen Trockensubstanz

Asche	2.92
In heisser conc. HCl löslich	92.21
„ „ unlöslich	7.79
Gesamt-Stickstoff	18.87
Darunter: In heisser conc. HCl löslicher Stickstoff	16.39
„ „ unlöslicher Stickstoff	2.48

#### In 100 Teilen Gesamtstickstoff

In heisser conc. HCl löslicher Stickstoff	86.87
Darunter: { Ammoniak Stickstoff	2.52
{ Durch Phosphowolframsäure	
{ fallbarer Stickstoff	13.11
{ Stickstoff in anderer Form	71.24
In heisser conc. HCl unlöslicher Stickstoff	13.13

#### A. Monaminosäuren in den Spaltungsprodukten.

200 g. käufliche Sakusan-Seide wurden mit 600 c.c. Salzsäure (1.19) im Rückflusskühler zwei Tage gekocht; nach dem Erkalten wurde die Flüssigkeit mit Wasser verdünnt, vom unlöslichen Rückstand abfiltriert und im Vacuum stark eingeeengt.

Man versetzte nun dieselbe mit 500 c.c. absolutem Alkohol und leitete trockenes Salzsäure gas bis zur Sättigung ein, und dampfte wieder im



Vakuum ein. Diese Operation wurde nochmals wiederholt. Als man den so bereiteten dicken Syrup mit einigen Krystallen von salzsaurem Glykokoll ester geimpft und in kaltem Zimmer einige Tage stehen liess, schieden sich die Krystalle von salzsaurem Glykokoll ester in reichlicher Menge aus, die abgesaugt, mit absolutem Alkohol und Aether gewaschen und getrocknet wurden. Aus der Mutter lauge schieden sich noch kleine Mengen in zweiter Ernte aus. Die Gesamtausbeute betrug 16.4 g. Diese Krystalle wurden aus Aethylalkohol umkrystallisiert. Sie bestanden aus farblosen Prismen und schmolzen bei  $145^{\circ}$  (uncorr.). Sie wurden im Vakuum bei  $100^{\circ}$  getrocknet und analysiert.

0.3225 g Subst		0.08136 g Cl
		Cl
$C_4H_{10}O_2N.Cl$	Ber.	25.45
	Gef.	25.23

Die Mutter lauge von Salzsäurem Glykokoll ester wurde im Vakuum eingedampft und aus derselben nach der bekannten Ester Methode von E. Fischer die freien Aminosäureestern dargestellt, und bei niederem Druck fraktioniert. Nach der Verseifung der einzelnen Ester fraktionen haben wir folgende Aminosäuren isoliert.

Glykokoll	1.1 g
Alanin	8.4
Alanin + Leucin	2.0
Asparaginsäure	1.7
Glutaminsäure	?
Prolin	Weinig
Tyrosin (aus dem undestillierbaren Rückstand)	2.4

#### Analyse des Alanin präparates

0.3995 g Subst		0.06411 g N
		N
$C_3H_7NO_2$	Ber.	15.73
	Gef.	16.04

Die Menge des Leucins reichte zur Analyse nicht hin.

Asparaginsäure wurde als Kupfersalz gereinigt. Es bestand aus langen blauen Nadeln, ziemlich schwer löslich in Wasser und schmolz bei  $218^{\circ}$  (uncorr.) Aus dem Kupfersalz wurde freie Asparaginsäure dargestellt. Sie schmolz bei  $228^{\circ}$  (uncorr.)

Das bei  $100^{\circ}$  getrocknete Präparat gab 10.47% N. (Auf  $C_4H_7NO_4$  berechnet 10.53% N.).

Nach Serin und Glutaminsäure wurde vergebens gesucht.

Zur Isolierung des Tyrosins wurde 100 g. Sakusan-Seide mit 300 c.c. conc. HCl zwei Tage gekocht, vom unlöslichen Rückstand abfiltriert und im Vacuum eingedampft, um den grössten Teil der Salzsäure auszutreiben. Der Rückstand wurde mit Wasser verdünnt, mit Natronlauge neutralisiert, mit Tierkohle entfärbt und einige Tage stehen gelassen. Das ausgeschiedene Tyrosin wurde abgesaugt, mit Wasser, Alkohol und Aether gewaschen. Die Ausbeute betrug 1.4 g. = 1.4% der trockenen Seide.

Organische Basen.

100 g. luft trockener Substanz (=86.84 g. Trockensubstanz) wurden mit 300 c.c. HCl (1.19) zwei Tage gekocht. Nach dem Erkalten wurde die Flüssigkeit mit Wasser verdünnt und mit Phosphorwolframsäure gefällt. Der Niederschlag wurde in bekannter Weise durch Baryt selegt. Die so erhaltene stark alkalische Flüssigkeit wurde mit Kohlensäure gesättigt und mit Quecksilberchlorid versetzt. Aus dem Quecksilberchlorid-Niederschlag wurde das *salzsaure Salz des Histidins* (entsprechend 2.4 g. freies Histidin) dargestellt.

Aus dem salzsauren Salz wurde Histidin Silber bereitet, indem das Salz in Wasser gelöst, durch Silbernitrat von Salzsäure befreit und zum klaren Filtrat Silber nitrat und Ammoniak zugegeben wurde, wobei ein weisser Niederschlag von Histinsilber entstand, der mit Wasser, Alkohol und Aether gewaschen, bei  $100^{\circ}$  getrocknet und analysiert wurde.

0.211 Ig Subst

0.122 g Ag.

Ag

$C_6H_7N_3O_2Ag_2$

Ber.

58.53

Gef.

57.82

Aus dem salzsauren Salz wurde durch Zusatz von Natrium pikrat das Histidin pikrat als gelbe Prismen erhalten; es schmolz bei 80-90° zu einem Oel.

Das Filtrat von Quecksilberchlorid-Niederschlag wurde durch Schwefelwasserstoff von Silber befreit und im Vakuum eingedampft, um den Schwefelwasserstoff auszutreiben und mit Silbernitrat und Baryt in kleinem Ueberschuss versetzt. Aus dem dabei entstandenen Niederschlag wurde kein Arginin isoliert. Dagegen hat man aus dem Filtrate von Silbernitrat und Baryt-Niederschlag in bekannter Weise 6.2 g. *Arginin pikrat* (= 2.7 g. Arginin) isoliert.

Das Arginin pikrat wurde mehreremal aus heissem Wasser umkrystallisiert und im Vakuum bei 100° getrocknet und analysiert.

0.4725 g Subst.	0.2670 g Pikrinsäure				
0.1650 g „	0.2152 g CO <sub>2</sub>	0.0510 g H <sub>2</sub> O			
0.1690 g „	36.5 c. N (20° 753 <sup>mm</sup> )				
	C	H	N	Pikrinsäure	
C <sub>6</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>	Ber.	35.73	4.22	24.32	56.82
	Gef.	35.57	3.42	24.17	56.51

Aus dem Pikrate wurde das methylester salzsaure Salz des Arginins dargestellt; farblose Prismen, Schmolzen bei 175—180° (uncorr.) unter lebhaftem Schäumen. Für die Analyse war es im Vacuum bei 100° getrocknet.

0.1065 g Subst	0.0280 g Cl	
	Cl	
C <sub>7</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub> · 2HCl	Ber.	27.20
	Gef.	26.29

Das Nitrat des Arginins:—farblose Prismen F. P. 155—165°.

## II.—Yamamai-Seide. (Antheraea Yamamai).

Die kältliche Yamamai-Seide hat folgende Zusammensetzung.

Wasser	11.29
Trockensubstanz	88.71

## In 100 Teilen Trockensubstanz

Asche	4.73
In heisser conc. HCl löslich	97.07
„ „ unlöslich	2.93
Gesamt-Stickstoff	17.73
Darunter: In heisser conc. HCl löslicher Stickstoff	17.26
„ „ unlöslicher Stickstoff	0.47

## In 100 Teilen Gesamt-Stickstoff

In heisser conc. HCl löslicher Stickstoff	97.34
Darunter: { Ammoniak Stickstoff	3.85
{ Durch Phosphowolfsäure	
{ fällbarer Stickstoff	19.44
{ Stickstoff in anderer Form	74.05
In heisser conc. HCl unlöslicher Stickstoff	2.66

*Montminosäuren.*

200 g. Yamamai-Seide wurden mit 600 c.c. Salzsäure (1.19) 30 Stunden gekocht<sup>1</sup> und nach der Ester methode die folgenden Aminosäuren dargestellt.

Glycocol	11.1 g
Alanin	12.7
Alanin + Lencin	2.3
Asparagin säure	2.3
Glutaminsäure	1.2
Rückstand	2.6

## Analyse der Aminosäuren.

1. Glycocollester-salzsaure Salz. Aus heissem Alkohol unkry- stallisiert Schmolz es bei 145° (uncorr.) Für die Analyse wurde es im Vakuum bei 100° getrocknet.

1. Die Yamamai Seide ist gegen Säuren und Alkalien viel beständiger als andere Seidenarten.

0.3110 g Subst		0.0806 g Cl
		Cl
$C_4H_{10}NO_2Cl$	Ber.	25.45
	Gef.	25.94

## 2. Alanin.

0.400 g Subst		0.06262 g N
		N
$C_3H_7NO_2$	Ber.	15.73
	Gef.	15.66

3. Asparaginsäure-Kupfersalz; bestand aus langen Nadeln oder Prismen, der Schmelzpunkt war  $218^\circ$  (uncorr.)

0.232 g. Subst (über Schwefelsäure bei gewöhnlicher Temperatur getrocknet) verlor bei  $110^\circ$  0.0375 g. Wasser.

0.2485 g Subst (Wasser frei) gab	0.0934 g CuO
0.1925 g „ „	0.01491 g N

		Krystall wasser	
$C_4H_5NO_4Cu + 2H_2O$	Ber.	15.45	
	Gef.	16.66	
		N	Cu
$C_4H_5NO_4Cu$	Ber.	7.19	32.82
	Gef.	7.50	30.06

Die Analyse stimmt nicht ganz gut, wahrscheinlich in Folge von Verunreinigung. Zur weiteren Reinigung genügte das Material nicht.

Das Vorhandensein von Prolin war zweifelhaft. Nach Serin und Phenylalanin wurden vergebens gesucht.

In der Mutter lauge von Asparaginsäure konnten wir kleine Mengen Glutaminsäure nachweisen; sie reagierte stark sauer und bildete schöne prismatische Krystalle von salzsaurem Salz, das in conc. Salzsäure schwer löslich war; sie bildete auch schwer lösliches Kupfersalz. Zur weitere Forschung reichte die Menge nicht aus.

Aus dem undestillierbaren Rückstand wurde durch Verseifen mit Baryt 2.6 g. Tyrosin isoliert.

Zur Gewinnung des Tyrosins wurden 20 g. Rohmaterial mit Schwefelsäure gekocht und in bekannter Weise haben wir 0.4 g. Tyrosin isoliert. Das Rohprodukt wurde einmal aus Wasser umkrystallisiert und analysiert.

0.320 g Subst		0.22485 g N
		N
$C_9H_{11}NO_2$	Ber.	7.93
	Gef.	8.10

#### *Organische Basen.*

Es wurde bloss die quantitative Analyse der organischen Basen ausgeführt. Zu diesem Zwecke wurden 20 g. Rohmaterial mit conc. Salzsäure drei Tage gekocht, und der salzsaure Extrakt wurde mit Phosphorwolframsäure gefällt. Der Niederschlag wurde in bekannter Weise durch Baryt zerlegt. Die stark alkalische Flüssigkeit, die freie Basen enthielt, wurde mit Kohlensäure gesättigt und mit einer wässrigen Quecksilberchlorid lösung versetzt. Der Quecksilber chlorid-Niederschlag wurde unmittelbar zur Stickstoff-Bestimmung nach der Kjeldahl'schen Methode angewendet. . . *Histidin stickstoff*.

Das Filtrat vom Quecksilberchlorid-Niederschlag wurde durch Schwefelwasserstoff von Quecksilber befreit und im Vakuum eingedampft. Als der Schwefelwasserstoff vollständig ausgetrieben war, wurde die Flüssigkeit mit Silbernitrat versetzt, vom gebildeten Silberchlorid adfiltriert und zum Filtrat wurde Silber nitrat und Baryt im Ueberschuss zugegeben. Der braune Niederschlag enthält Arginin. . . *Arginin stickstoff*. . .

Das Filtrat von Silbernitrat und Baryt-Niederschlag wurde nach dem Entfernen des Silbers und des Baryts wieder mit Schwefelsäure angesäuert und mit Phosphorwolframsäure versetzt. Der Niederschlag wurde gleich zur Stickstoff-Bestimmung nach Kjeldahl angewendet. . . *Lysin stickstoff*.

Es ist zu bemerken, dass die Trennung von Arginin und Lysin nach der oben angegebenen Methode nur unvollkommen war. In

der Lysin fraktion haben wir immer nicht unbedeutende Mengen Arginin gefunden.

In 100 Teilen Trokensubstanz

Gesamt Stickstoff	17.73
Durch Phosphowolframsäure fällbarer Stickstoff	3.45
Histidin Stickstoff	0.37
Arginin Stickstoff	1.10
Lysin Stickstoff	1.26

Als freie Base berechnet

Histidin	1.37
Arginin	3.41
Lysin	6.57

Im nächsten Versuche haben wir die entbastete Yamamai-Seide analysiert. Zu diesem Zwecke wurden 20 g. Rohseide mit 5% Salzsäure eine Stunde gekocht, mit warmem Wasser gewaschen, dann wurde sie mit 10% Marseille seife 2 Stunden gekocht, abgepresst, und mit warmem Wasser gewaschen, nochmals mit 2% Natronlauge eine Stunde auf 50° erwärmt und mit Wasser gewaschen, getrocknet und analysiert. Es wurde gefunden.

In 100 Teilen Trockensubstanz

	Roh	Entbastet
Verlust beim Entbasten	—	27.30
In heisser conc. Salzsäure löslich	97.07	97.23
"  "  unlöslich	2.93	2.77
Gesamt Stickstoff	17.73	18.42

In 100 Teilen Gesamt-Stickstoff

Ammoniak stickstoff	3.85	6.20
Durch Phosphowolframsäure fällbarer Stickstoff	19.44	15.05
Stickstoff in anderer Form	74.05	78.75



Die Rohseide hat beim Entbasten 27% der Trockensubstanz verloren. Dieser Verlust besteht hauptsächlich aus anorganischer substanz, Leim u. a. Der prozentische Gehalt an Gesamtstickstoff ist durch Entbasten etwas erhöht. Der Basen stickstoff dagegen hat etwas abgenommen, bleibt jedoch immer noch höher als 15% der Gesamtstickstoff. Vergleicht man dies mit demjenigen des Seiden fibroins, nämlich nur 1.4% des gesamten Stickstoffes, so muss man annehmen, dass die Yamamai-Seide eine stark vom Seiden fibroin abweichende Zusammensetzung hat. Ausserdem sieht man aus der Analyse, dass die aussere Schichte der Yamamai-Seide viel reicher an Basenstickstoff ist als das Innere.

### III.—Seide. (*Bombyx mori*).

#### 1. Rohseide

Wasser	12.90
Trockensubstanz	87.10

#### In 100 Teilen Trockensubstanz

Asche	0.63
In heisser conc. HCl löslich	99.14
„ „ unlöslich	0.86
Gesamt-Stickstoff	18.98
Darunter: In heisser conc. HCl löslicher Stickstoff	18.86
„ „ unlöslicher „	0.12

#### In 100 Teilen Gesamt stickstoff

In heisser conc. HCl löslicher	99.34
Darunter: Ammoniak Stickstoff	4.57
Durch Phosphowolframsäure	
fällbarer Stickstoff	1.78
Stickstoff in anderer Form	92.98
In heisser conc. HCl unlöslicher Stickstoff	0.66

#### 2. Entbastete Seide

Die künstliche Rohseide der besten Sorte wurde mit 10% Marseilleseife 2 Stunden bei 60-65° gekocht und mit Wasser gewaschen. . . (A)

(A) Wurde nochmals mit 10% Marseille Seife 2 Stunden gekocht, mit Wasser gewaschen, dann mit Wasser gekocht und mit 0.1% Natronlauge erwärmt und mit Wasser sorgfältig gewaschen... (B)

In 100 Teilen Trockensubstanz

	Rohseide	Entbastete Seide	
		(A)	(B)
Verlust beim Entbasten	—	16.0	37.0
In conc. Salzsäure löslich	99.14	99.33	ungefähr 100
„ „ unlöslich	0.86	0.67	spur
Gesamt stickstoff	18.98	17.83	17.97

In 100 Teilen Gesamt stickstoff

Ammoniak stickstoff	4.57	4.71	5.85
Durch Phosphowolframsäure			
fällbarer Stickstoff	1.78	1.38	1.47
Stickstoff in anderer Form	92.99	93.91	92.68

Aus den obigen Resultaten sieht man, dass es kein merkbarer Unterschied zwischen Roher und entbasteter Seide gibt, bloss ist der Gesamt stickstoff und der Basen stickstoff in der entbasteten Seide etwas kleiner als in der Rohseide. Doch nimmt der Basenstickstoff nie so stark ab, dass man ihn nicht als eigentlichen Bestandteile des Fibroins betrachten dürfte.

Wir haben aus der Rohseide 12% Tyrosin isoliert.

#### IV.—Kuriwata. (*Caligula japonica*).

Die käufliche Kuriwata Seide hatte folgende Zusammensetzung

Wasser	11.71
Trockensubstanz	88.29

In 100 Teilen Trockensubstanz

Asche	3.85
In heisser conc. HCl löslich	88.34
„ „ unlöslich	11.66

Gesamt-Stickstoff	16.73
Darunter. In conc. HCl löslicher Stickstoff	15.77
„ „ unlöslicher Stickstoff	0.96
In 100 Teilen Gesamt stickstoff	
In conc. HCl löslicher Stickstoff	94.26
Darunter Ammoniak-Stickstoff	4.08
Durch Phosphorwolframsäure	
fällbarer Stickstoff	15.54
Stickstoff in anderer Form	74.64
In conc. HCl unlöslicher Stickstoff	5.74

200 g. Roh-Kuriwata wurden mit kalter 5% iger Salzsäure 20 stunden digeriert und mit Wasser gewaschen, dann wurde sie mit 10% Marseille seife 2 Stunden gekocht, stark abgepresst, mehreremal mit heissem destilliertem Wasser gewaschen und getrocknet. Der Verlust an Gewicht betrug 5.33% der Trockensubstanz.

200 g. der so behandelten Seide wurden mit 700 c.c. conc. Salzsäure (1.19) 8 Stunden gekocht. Nach dem Erkalten wurde die Flüssigkeit mit Wasser verdünnt und abfiltriert.

In heisser conc. HCl gelöst	87.28%
Der unlösliche Rückstand	12.72%

Aus dem salzsauren Extrakt wurde in bekannter Weise die freien Aminosäuren dargestellt. Es wurden isoliert.

Glycocoll ester HCl salz	24.49 g	(=13.12 g Glycocol)
Alanin	25.08	
Leucin	14.05	
Prolin	{	Actives 1.06
		Racemisches 0.48
Phenylalanin		0.95
Asparaginsäure		0.30
Tyrosin (aus 100 g)		4.87

Analyse der Aminosäuren.



Das Vorhandensein von Prolin wurde durch seinen eigentümlichen Geruch, durch seine Löslichkeit in absolutem Alkohol und besonders durch sein in Alkohol lösliches Kupfersalz sicher festgestellt, aber die Analyse gab kein befriedigendes Resultat, weil es von anderen Aminosäuren Kupfersalz schwer zu trennen war und zur weiteren Reinigung genügte die Menge nicht.

Phenylalanin und Asparaginsäure waren auch nicht rein dargestellt; wir haben bloss das Phenylalanin durch sein charakteristisches salzsaure Salz und die Löslichkeit seines Esters in Ather identifiziert.

Asparaginsäure wurde analysiert und wir haben den Stickstoff gehalt etwa um 1% höher gefunden, so dass es noch mit anderen Aminosäuren verunreinigt gewesen sein muss. Von den ziemlich starken sauren Eigenschaften und dem charakteristischen Kupfersalz zu urteilen, zweifeln wir kaum, dass es Asparaginsäure war.

Nach Glutaminsäure wurde mit besonderen Vorsicht gesucht; kein positives Resultat konnten wir jedoch erzielen.

#### *Organische Basen.*

Auf die Isolierung der organischen Basen haben wir verzichtet und nur die quantitative Analyse ausgeführt. Es wurde gefunden.

	In 100 Teilen Trockensubstanz.	In 100 Teilen Gesamtstickstoff
Gesamt stickstoff	16.73	100.
Durch Phosphowolframsäure fällbarer		
Stickstoff	2.62	15.54
Darunter: { Histidin stickstoff	0.27	1.61
{ Arginin stickstoff	0.56	3.35
{ Lysin stickstoff	0.47	2.81

Als freie Base berechnet (In 100 Teilen Trocken substanz)

Histidin	1.01
Arginin	1.74
Lysin	2.43

# CHEMISCHE ZUSAMMENSETZUNG DER VERSCHIEDENEN SEIDENARTEN.

	Bombyx.	Sakusan.	Yamamai.	Kuriwata.
	(Roh seide)	(Roh seide)	(Roh seide)	(Roh seide)
Wasser... ..	12.90	13.16	11.29	11.71
Trockensubstanz ... ..	87.10	86.84	88.71	88.29

## In 100 Teilen Trockensubstanz.

Asche ... ..	0.63	2.92	4.73	3.85
In heisser conc. HCl löslich ... ..	99.14	92.21	97.07	88.34
„ „ unlöslich ... ..	0.86	7.79	2.93	11.66
Gesamt stickstoff ... ..	18.98	18.87	17.73	16.73
Darunter. In conc. HCl löslicher stickstoff.	18.86	16.39	17.26	15.77
„ „ unlöslicher „	0.12	2.48	0.47	0.96

## In 100 Teilen Gesamt stickstoff.

In heisser conc. HCl löslicher Stickstoff.	99.34	86.87	97.34	94.26
Darunter. Ammoniak stickstoff ... ..	4.57	2.52	3.85	4.08
Durch Phosphowolframsäure				
fällbarer Stickstoff... ..	1.78	13.11	19.44	15.54
Stickstoff in anderer Form...	92.99	71.24	74.05	74.64
In heisser conc. HCl unlöslicher Stickstoff.	0.66	13.13	2.66	5.74

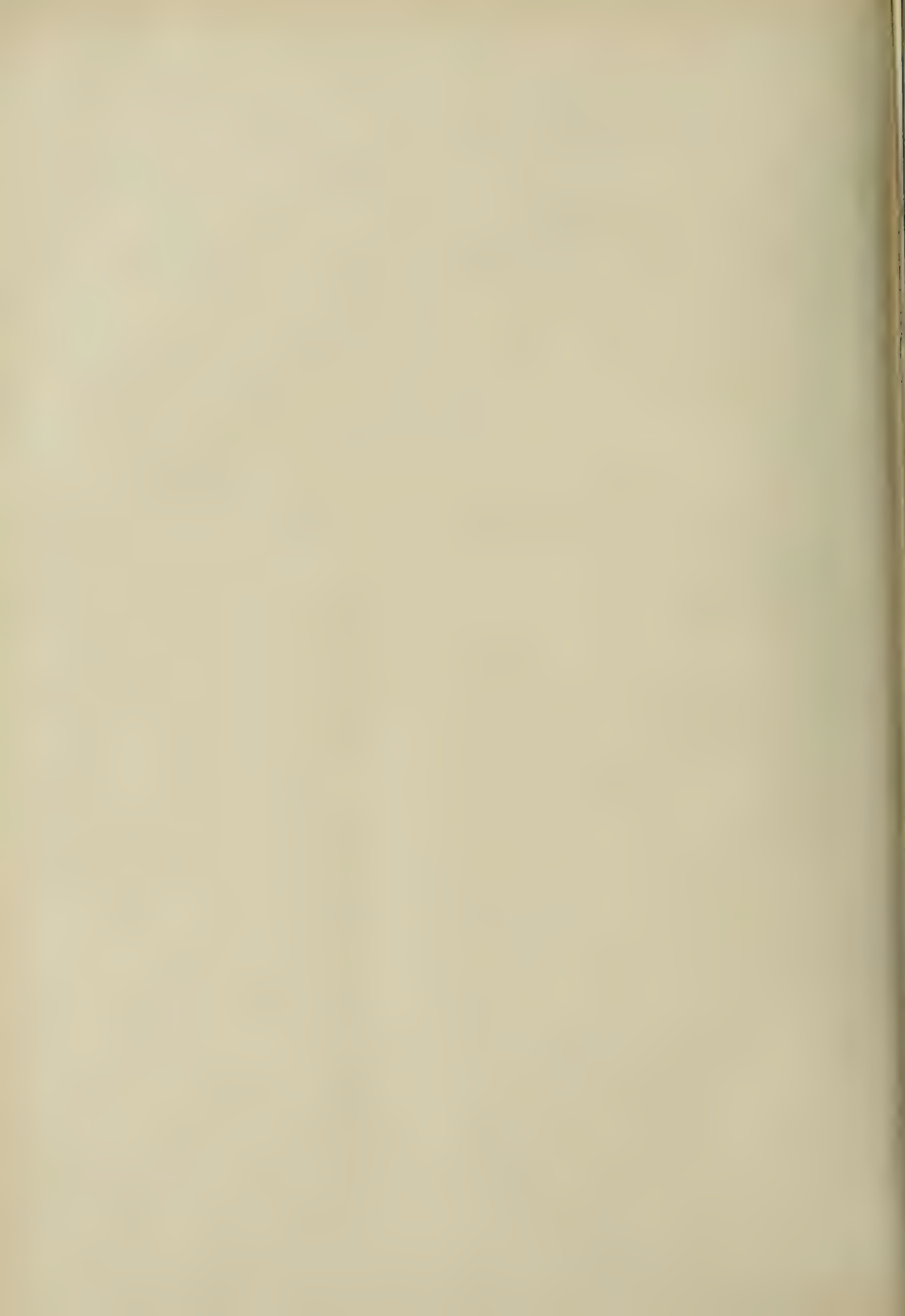
# SPALTUNGS PRODUKTE DER VERSCHIEDENEN SEIDEN ARTEN.

(Aus 100 g. Trockensubstanz.)

	Bombyx.		Sakusan.	Yamamai.	Kuriwata.	Spinnen
	Fibroin.	Sericin.	(Roh)	(Roh)	(Entlastet)	Seide. <sup>1</sup>
	(Fischer u. Skita)					
Glykokoll...	36.0	0.1—0.2	5.7	6.3	7.7	35.13
d. Alanin ...	21.0	5.0	4.8	7.2	15.3	23.4
l. Lencin ...	1.5	+	1.2	1.3	7.95	1.76
Phenylalanin ...	—	—	—	+	+	—
Prolin ...	0.3	—	+	+	Activ. 4.0 Rac. 0.2 }	3.68
Glutaminsäure ...	—	—	+	0.6	?	6.16
Asparaginsäure ...	—	—	1.0	1.0	0.2	
Cystin ...	—	—	—	—	—	
Serin ...	1.6	6.6	?	?	?	
Oxyprolin ...	—	—	—	—	—	
l. Tyrosin ...	12.0	5.0	1.4	2.0	5.5	4.2
Histidin ...	+	—	2.7	1.6	1.01	
Arginin ...	1.0	4.0	3.1	3.8	1.74	5.24 <sup>2</sup>
Lysin ..	+	+	+	7.4	2.43	
Tryptophan ...	+	—	—	—	—	
Ammoniak ...	1.05	1.87	0.6	0.8	0.8	1.16
Valin...	+	+	—	—	—	
Glykosamin ...	—	—	—	—	—	

<sup>1</sup> Spinnen seide enthält ausserdem 0.59%, Asche 0.66% Fettsäuren.<sup>2</sup> Diaminosäure, als Arginin berechnet.





# Ueber die Eiweissstoffe aus Reissamen.<sup>1</sup>

VON

U. Suzuki, K. Yoshimura und S. Fuji.

Der geschälte Reiskern besteht aus zwei Teilen, von denen die äussere Schichte, die Kleie ungefähr 15% ausmacht. Die chemische Zusammensetzung der Kleie ist von jener des entkleiten Reissamens sehr verschieden; man muss natürlich die beiden Teile für sich untersuchen.

In 100 Teilen Trockensubstanz

	Entkleiter Reis (Hakumai)	Kleie (Nuka)
Gesamt stickstoff	1.200	2.958
Eiweiss stickstoff	1.165	2.665
Nicht-Eiweiss stickstoff	0.035	0.293
Eiweiss (Eiweiss-N $\times$ 6.25)	7.282	16.655

Gesamt stickstoff als 100

Gesamt stickstoff	100.00	100.00
Eiweiss stickstoff	97.09	90.10
Nicht-Eiweiss stickstoff	2.91	9.90

Um die chemische Natur der Eiweisskörper in der Kleie und in dem entkleiten Reis zu vergleichen wurden je 10 g. getrocknete Probe mit 100 c.c. der folgenden Lösungsmitteln versetzt und bei Zimmertemperatur stehen gelassen. Nach 24 Stunden abfiltriert; mit dem Filtrate wurde die Stickstoffbestimmung nach der Kjeldahl'schen Methode ausgeführt.

In 100 Teilen Trockensubstanz wurde stickstoff gelöst:

Lösungsmittel	Entkleiter Reis	Kleie
Destilliertes Wasser	0.07 g	0.65 g

1. Diese Abhandlung ist schon in the Journal of the Tokyo Chemical Society Vol XXIX No. 3. (March 1908), publiziert worden. Später ist eine vorläufige Mitteilung über Reisprotein von Rosenheim, O und Kajura S. erschienen (The Proteins of Rice:—Proc. Physiol. Soc. 1908, Bd 54, u Journ. of Physiol. 1808. Bd 36. No 6).

60% Alkohol	0.11	0.16
10% NaCl	0.17	1.37
0.2% NaOH	0.85	1.51
0.3% „	—	2.36
0.4% „	—	2.49

## Gesamt stickstoff als 100

Lösungs mittel	Entkleiter Reis	Kleie
Destilliertes Wasser	5.84	17.45
60% Alkohol	9.17	4.38
10% NaCl	14.17	36.87
0.2% NaOH	70.90	40.59
(0.3% „ )	—	(63.42)
(0.4% „ )	—	(67.02)

Wie man sieht, enthält der entkleite Reis nur sehr wenig Eiweisskörper, die in Wasser, Alkohol und 10% Natriumchlorid löslich sind. Der grösste Teil besteht aus denjenigen Eiweisskörpern, die in verdünnter Natronlauge löslich sind. Die Kleie enthält noch etwas mehr in Natriumchlorid lösliches Eiweiss (Globulin).

*Darstellung der Eiweisskörper.*

Wie oben erwähnt, ist das Eiweiss im entkleiten Reis und in der Kleie grösstenteils in verdünnter Natronlauge löslich; so haben wir versucht, nach Ritthausen'scher Methode das Eiweiss zu extrahieren. Zu diesem Zwecke wurde das getrocknete, fein gepulverte Material (die Kleie vorher entfettet) mit 0.2% Natronlauge mazeriert, nach 24 Stunden mit dem Tuch koliert. Das Filtrat wurde mit verdünnter Essigsäure schwach angesäuert, wobei ein dicker, weisser Niederschlag in reichlicher Menge entstand, der gesammelt, mit kaltem Wasser, Alkohol und zuletzt mit Aether gewaschen wurde. Das so bereitete Rohprodukt wurde nochmals in verdünnter Natronlauge gelöst und mit Essigsäure gefällt. Wenn man mit wenig Material arbeitet, kann man aus dem entkleiten Reis etwa 70% des gesamten Eiweiss und aus der Kleie etwa 50% gewinnen. Wenn

man aber mit grösserer Menge arbeitet, so wird die Ausbeute schlechter.

Das in oben erwähnter Weise dargestellte Eiweisspräparat aus dem entkleiten Reis war gelblich braun gefärbt, während jenes der Kleie dunkel gefärbt und viel unreiner als der Ersteres war. Beide Eiweisspräparate hatten folgende Zusammensetzung.

	Eiweiss aus	
	dem entkleiten Reis	der Kleie
Asche	0.02	2.10
In heisser conc. Salzsäure löslich	98.00	89.24
„ „ unlöslich	2.00	10.76
Gesamt stickstoff	16.54	12.49
Darunter: In heisser Salzsäure löslicher		
Stickstoff	16.23	11.34
„ „ unlöslicher		
Stickstoff	0.31	1.14
Ammoniac stickstoff	1.92	0.93
Durch Phosphowolframsäure fällbarer Stickstoff		
( $\text{NH}_3\text{-N}$ ausgenommen)	4.91	4.05
Stickstoff in anderer Form	9.40	6.34

Gesamt stickstoff als 100

In heisser Salzsäure löslicher Stickstoff	98.15	90.89
Darunter: { Ammoniac stickstoff	11.61	7.48
{ Durch Phosphowolframsäure		
{ fällbarer Stickstoff	29.67	32.57
{ Stickstoff in anderer Form	56.87	50.84
In heisser Salzsäure unlöslicher Stickstoff	1.85	9.11

I.—Das Eiweiss aus dem entkleiten Reis.

A. Monamino-säuren.

250 g. getrocknetes Eiweiss wurden mit 750 c.c. Salzsäure (1.19) 8 Stunden gekocht. Nach dem Erkalten wurde die Flüssigkeit vom unlöslichen Rückstand abfiltriert und im Vacuum zur Trocknung eingedampft, mit absolutem Alkohol übergossen und trockenes Salzsäure gas

bis zur Sättigung eingeleitet. Nach dem diese Operation nochmals wiederholt war, wurde die Flüssigkeit stark eingedampft, und mehrere Tage in kaltem Zimmer stehen gelassen. Da aber kein Glykokollster-salzsäure Salz ausgeschieden war, wurde die Flüssigkeit in bekannter Weise in die freien Aminosäurenestern verwandelt und bei niederem Druck fraktioniert.

Nach der Verseifung der einzelnen Fraktionen wurden folgende Aminosäuren isoliert:

Alanin	9.2 g
Leucin	35.8
Phenylalanin	4.9
Asparaginsäure	1.0
Actives Prolin (Kupfersalz)	7.5
Racemisches Prolin ( „ )	3.0

#### Analysen der Aminosäuren

##### 1. Alanin

0.1400 g Subst	0.201 g CO <sub>2</sub>	0.0988 g H <sub>2</sub> O		
0.188 g „	22.0 cc N (13° 754mm)			
	C	H	N	
C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>	Ber.	40.45	7.86	15.73
	Gef.	39.16	7.84	16.20

Die Analyse stimmt nicht gut überein; wahrscheinlich durch kleiner Menge Glykokoll verunreinigt.

##### 2. Leucin

0.1235 g Subst	0.2458 g CO <sub>2</sub>	0.1075 g H <sub>2</sub> O		
	C	H		
C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>	Ber.	54.63	9.85	
	Gef.	54.54	9.67	

##### 3. Asparaginsäure

0.129 g Subst	11.1 cc N (11° 754mm)			
	N			
C <sub>4</sub> H <sub>7</sub> NO <sub>4</sub>	Ber.	10.52		
	Gef.	10.28		

## 4. Phenylalanin

0.1867 g Subst	0.4458 g CO <sub>2</sub>	0.1135 g H <sub>2</sub> O		
0.3304 g „	23.0 <sup>c.c.</sup> N (6. <sup>o</sup> 765 <sup>mm</sup> )			
		C	H	N
C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>	Ber.	65.42	6.66	8.40
	Gef.	65.12	6.75	8.53

Das Phenylalanin bildete ein Pikrat, das aus gelben Nadeln bestand und bei 170—173° unter lebhaftem Schäumen schmolz. Diese Beobachtung stimmt mit der Beschreibung von Mayeda<sup>1</sup> vollständig überein.

Das Phenylalanin gab auch eine weisse Fällung mit Phosphowolframsäure in saurer Lösung. Es bildete ferner das charakteristische salzsaure Salz.

## 5. Prolin

Aktives Prolin kupfer (in Aethyl alkohol löslich)

0.1622 g Subst	0.0445 g CuO		
0.1900 g „	15.7 <sup>c.c.</sup> N (10. <sup>o</sup> 763 <sup>mm</sup> )		
		N	Cu
(C <sub>5</sub> H <sub>8</sub> NO <sub>2</sub> ) <sub>2</sub> Cu	Ber.	9.60	21.81
	Gef.	10.00	21.81

Das in Aethylalkohol unlösliche, racemische Prolin kupfer war nach mehrmaliger Umkrystallisation immer noch unrein und hat kein befriedigendes Resultat gegeben.

Nach Valin und Isoleucin haben wir mit besonderer Sorgfalt gesucht ohne ein positives Resultat zu bekommen.

## B. Tyrosin, Leucin und Glutaminsäure.

100 g. trockenes Eiweiss wurden mit 600 c.c. 25% Schwefelsäure im Rückflusskühler 20 Stunden gekocht. Nach dem Entfernen der Schwefelsäure durch Baryt wurde die Flüssigkeit im Vakuum stark eingedunstet, wobei das Tyrosin als weisse Nadeln sich ausschieden, die aus heissem

1. Mayeda, Zeitsch. f. Physiol. Chem. 51: 263

Wasser umkrystallisiert wurden. Ausbeute 0.5 g. Diese Krystalle gaben starke Millon'sche Reaktion.

0.1908 g Subst		0.01499 g N
		N
$C_9H_{11}NO_3$	Ber.	7.74
	Gef.	7.82

Die Mutter lauge von Tyrosin lieferte nach weiterem Einengen 8.1 g. Lencin, welches aus heissem Wasser umkrystallisiert und analysiert wurde.

0.2092 g Subst		0.0230 g N
		N
$C_6H_{13}NO_2$	Ber.	10.69
	Gef.	10.99

Es wurde ferner das Kupfersalz des Lencins dargestellt und analysiert.

0.1868 g Subst		0.047 g CuO
		Cu
$(C_6H_{12}NO_2)_2 Cu$	Gef.	19.62
	Gef.	20.10

Die Mutter lauge von Lencin wurde weiter bis auf 100 c.c. eingengt und trockenes Salzsäuregas bis zur Sättigung eingeleitet und zwei Tage mit Eis abgekühlt, wobei die farblose Nadeln von salzsaurer Glutaminsäure in reichlicher Menge sich ausschieden. Die Ausbeute betrug 18.1 g. (= 14.5 g. Glutaminsäure).

Das gereinigte Salz schmolz bei  $203^\circ$ — $204^\circ$  (uncorr.)

0.4379 g Subst		0.03347 g N	
0.3765 g „		0.07512 g Cl	
		N	Cl
$C_6H_9NO_4 HCl$	Ber.	7.63	19.34
	Gef.	7.64	19.95



Zur Darstellung freier Glutaminsäure wurde das salzsaure Salz in wenig Wasser gelöst, mit Natronlauge neutralisiert und erkalten gelassen. Es schieden sich dabei glänzende rhombische Krystalle aus, die bei 205—210° schmolzen. Für die Analyse wurden sie bei 100° getrocknet.

0.2200 g Subst		0.02161 g N
		N
$C_5H_9NO_4$	Ber.	9.52
	Gef.	9.82

Aus 100 g. Eiweiss wurden isoliert.

Tyrosin	0.5 g
Leucin	8.1
Glutaminsäure	14.5

### C. Organische Basen.

Das Eiweiss präparat wurde mit conc. Salzsäure 8 Stunden gekocht und mit Phosphowolframsäure gefällt. Mit dem dabei entstandenen Niederschlag wurde die Hexonbasen-Bestimmung in bekannter Weise nach der A. Kossel'schen Methode ausgeführt. Es wurden gefunden.

In 100 Teilen Trockensubstanz

Durch Phosphowolframsäure fällbarer Stickstoff ( $\frac{NH_3 \cdot N}{\text{ausgen.}}$ ) 4.91

Darunter:	{	Histidin stickstoff	0.57	} 4.72
		Arginin stickstoff	2.24	
		Lysin stickstoff	1.91	

Als freie Base berechnet

{	Histidin	2.12	} 19.02
	Arginin	6.95	
	Lysin	9.95	

Isolierung der organischen Basen.

200 g. getrocknetes Eiweiss präparat wurden mit 1200 c.c. 25% Schwefelsäure 20 Stunden gekocht, mit Phosphowolframsäure gefällt.

Der Niederschlag wurde in bekannter Weise mit Baryt zerlegt. Die alkalische Flüssigkeit, die freie Basen enthielt, wurde mit Kohlensäure gesättigt und mit Quecksilberchlorid versetzt. Der weisse Niederschlag wurde mit Schwefelwasserstoff zerlegt, vom Schwefelquecksilber abfiltriert, stark eingeeengt und im Exikator stehen gelassen. Nach einiger Zeit schied sich das salzsaure Histidin als farblose Prismen oder Platten aus, die 2.2 g. betrug (= 1.6 g. Histidin). Es schmolz bei 228°. Für die Analyse wurde das gereinigte Präparat über Schwefelsäure getrocknet.

0.1094 g Subst	0.0178 g Cl
	Cl
$C_6H_9N_3O_2 \cdot HCl + H_2O$	Ber. 16.94
	Gef. 16.27

Das Filtrat vom Quecksilberchlorid-Niederschlag wurde durch Schwefelwasserstoff vom Quecksilber befreit, im Vakuum eingedampft, um Schwefelwasserstoff auszutreiben und die vorhandene Salzsäure durch Silbernitrat befreit und zum Filtrat wurde Silbernitrat und Baryt in kleinem Ueberschuss zugegeben. Der braune Niederschlag von Arginsilber wurde durch Schwefelwasserstoff zerlegt, im Vacuum verdampft, mit Salpetersäure neutralisiert, und mit einem Ueberschuss von Kupferoxydhydrat gekocht. Das dunkel blaue Filtrat wurde stark eingeeengt und im Exikator stehen gelassen. Es schieden sich dabei die dunkelblaue Nadeln von Arginin kupfernitrat aus, die mit 50% Alkohol, absolutem Alkohol und zuletzt mit Aether gewaschen wurden. Die Ausbeute betrug 5.4 g.

Das Kupfer salz verlor das Krystall wasser bei 112° und zerstörte sich bei 230—232° (uncorr.)

0.211 g Subst verlor bei 120°	0.0194 g Wasser	
		Krystall wasser
$(C_6H_{14}N_4O_2)_2 \cdot Cu (NO_3)_2 + 3H_2O$	Ber.	9.15
	Gef.	9.21

0.2017 g (Wasser freies Präparat)	0.0297 g CuO
	Cu
$(C_6H_{14}N_4O_2)_2 \text{ Cu } (NO_3)_2$	Ber. 11.87
	Gef. 11.77

Für die Bestimmung der Salpetersäure im Kupfer salze wurde es in Wasser gelöst, durch Schwefelwasserstoff zerlegt, vom Schwefelkupfer abfiltriert, und stark eingengt. Mit der so bereiteten Flüssigkeit wurde die Bestimmung nach der Nitron methode ausgeführt. Es wurde gefunden.

0.1857 g (Wasser freies Präparat)	0.0432 g $HNO_3$
	$HNO_3$
$(C_6H_{14}N_4O_2)_2 \text{ Cu } (NO_3)_2$	Ber. 23.52
	Gef. 23.21

Das Filtrat vom Arginin silber wurde durch Salzsäure und Schwefelsäure vom Silber und Baryt befreit, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Aus dem Phosphowolframsäure-Niederschlag wurde in bekannter Weise das Lysin pikrat dargestellt. Es bestand aus gelblichbraunen Prismen. Die Ausbeute betrug 4.4 g. (= 1.7 g. Lysin).

0.3520 Subst	0.2135 g Pikrinsäure
	Pikrinsäure
$C_6H_{14}N_2O_2 \cdot C_6H_3N_3O_7$	Ber. 61.07
	Gef. 60.65

Aus dem Pikrate wurde das salzsaure Salz dargestellt. Es bestand aus farblosen Prismen.

0.1122 g Subst	0.0245 g Cl
	Cl
$C_6H_{14}N_2O_2 \text{ CHI}$	Ber. 19.45
	Gef. 20.09

Aus 100 g. trockenem Eiweiss wurden isoliert.

Histidin	0.81 g
Arginin	1.60
Lysin	0.86

## II.—Das Eiweiss aus der Kleie.

### A. Tyrosin, Leucin und Glutaminsäure.

100 g. Eiweiss aus der Kleie wurde mit 25% Schwefelsäure hydrolysiert und in oben erwähnter Weise Tyrosin, Leucin und Glutaminsäure isoliert. Es wurde gefunden.

Tyrosin	0.3 g
Leucin	8.6 g
Glutaminsäure	4.7 g

1. Tyrosin gab starke Millon'sche Reaktion. Für die Analyse reichte die Menge nicht aus.

2. Leucin.

0.2002 g Subst	0.02195 g N
	N
$C_6H_{13}NO_2$	Ber. 10.69%
	Gef. 10.96%

3. Glutaminsäure salzsaures Salz. Schmelzpunkt  $202 - 204^\circ$  (uncorr.)

0.1709 g Subst	0.01249 g	N
0.2518 g „	0.04750 g	Cl
	N	Cl
$C_5H_9NO_4HCl$	Ber. 7.63	19.34
	Gef. 7.31	18.85

### B. Organische Basen.

In 100 Teilen Trockensubstanz

Durch Phosphowolframsäure fällbarer Stickstoff 4.06

Histidin Stickstoff	0.45	} 2.97
Arginin Stickstoff	1.55	
Lysin Stickstoff	0.97	

Als freie Basen berechnet

Histidin	1.68	} 11.54
Arginin	4.80	
Lysin	5.06	

Isolierung der organischen Basen.

100 g. trockenes Eiweiss wurden mit 600 c.c. 25% Schwefelsäure 20 Stunden gekocht und in bekannter Weise die Hexonbasen dargestellt.

1. Salzsäures Histidin:—Ausbeute 1.2 g. (= 0.88 g. Histidin). Im Kapillarrohr erhitzt schmolz es bei 240—253° und zersetzt sich gleich darauf.

0.1919 g Subst		0.03849 g N
0.2007 g „		0.03415 g Cl
	N	Cl
$C_6H_9N_3O_2HCl + H_2O$	Ber.	20.04
	Gef.	20.05
		16.94
		17.01

2. Arginin nitrat:—Feine weisse Nadeln. Ausbeute 2.2 g. (1.6 g. Arginin).

0.1664 g Subst	gab	0.0417 g $HNO_3$ (Nitron methode)
		$HNO_3$
$C_6H_{14}N_4O_2HNO_3 + \frac{1}{2}H_2O$	Ber.	25.61
	Gef.	25.05
Arginin pikrat	Ausbeute	4.5 g (1.8 g Arginin)
0.3358 g Subst		0.0265 g $H_2O$
0.2895 g „		0.1623 g Pikrinsäure
	$H_2O$	Pikrinsäure
$C_6H_{14}N_4O_2C_6H_3N_3O_7 + 2H_2O$	Ber.	8.20
	Gef.	7.89
		56.82
		56.07

Arginin Kupfernitrat:—Dunkel grüne Nadeln.

0.1839 g Subst	0.0177 g $H_2O$		
0.1839 g „	0.0243 g CuO		
		$H_2O$	CuO
$(C_6H_{14}N_4O_2)_2 Cu (NO_3)_2 + 3H_2O$	Ber.	9.16	10.75
	Gef.	9.62	10.56

3. Lysin pikrat reichte zur Analyse nicht aus.

Aus 100 g. Eiweiss wurden isoliert.

Histidin	0.88
Arginin	3.40
Lysin	Wenig

*Gusammenfassung der Resultate.*

Aus 100 g Trocken substanz wurden isoliert.

	Eiweiss aus	
	dem entkleiten Reis	der Kleie
	(Hakumai)	(Nuka)
Glykokoll	Vorhanden ?	
Alanin	3.7	
Valin	?	
Leucin	14.3	8.6
Prolin	3.3	
Phenylalanin	2.0	
Asparaginsäure	0.4	
Glutaminsäure	14.5	4.7
Serin	—	
Tyrosin	0.5	0.3
Cystin	—	
Lysin	0.86	—
Histidin	0.81	0.88
Arginin	1.60	3.40
Ammonia	2.33	1.13
Tryptophan	—	—

# Ueber die chemische Zusammensetzung der Tamari-Schoyu.

VON

K. Yoshimura.

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Die so genannte Tamari-Schoyu ist eine Art Schoyu, welche meistens im Mittel und West Japan mit Vorliebe gebraucht wird. Auf die Bereitung derselben will ich hier nicht näher eingehen. Es sei nur erwähnt, dass man als Ausgangsmaterial bloss Soja-bohnen anwendet, während die gewöhnliche Schoyu aus gleichen Mengen Soja und Weizen hergestellt wird. Es ist deshalb wohl begreiflich, dass es zwischen den chemischen Zusammensetzungen beider Schoyu arten einen merkbaren Unterschied gibt.

Ueber die gewöhnliche Schoyu liegt eine ausführliche Abhandlung von T. Suzuki, K. Aso und H. Mitarai vor, welche im hiesigen Bulletin Vol. VII. No. 4 erschienen ist. Sie haben u. A eine Anzahl stickstoffhaltigen Verbindungen und auch organische Säuren, die während des Reifeprozesses der Maische gebildet werden, in reinem Zustande isoliert und die chemische Natur derselben festgestellt. Was nun die Tamari-Schoyu betrifft, so gibt es noch keine Versuche in dieser Richtung. Es war deshalb meine Aufgabe, zuerst die stickstoffhaltigen Körper zu isolieren und mit denen der Schoyu zu vergleichen. Die Tamari-Probe, die ich für diese Untersuchung anwendete, ist von einer Brauerei aus Mikawa bezogen. Die Maische wurde im Juli 1906 aus 10 Koku (1 Koku=1.78 Hektoliter) Soja, 580 Pfund Kochsalz und 7 Koku Wasser hergestellt und im März 1908 abgepresst. Die so gewonnene Tamari oder Pressaft betrug etwa 2.2 Koku. Diese Probe hatte folgende quantitative Zusammensetzung:

	Tamari.	Schoyu (nach. Suzuki u.A.)
Reaktion	Deutlich sauer.	Sauer.
Spezifisches Gewicht (bei 15°)	1.205	1.197





sigkeit wurde jetzt mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Der Phosphowolframsäure-Niederschlag wurde in bekannter Weise durch Baryt zerlegt. Mit der alkalischen Flüssigkeit, die freie Basen enthielt, wurde zuert eine quantitative Analyse ausgeführt. Es wurde gefunden.

	1 Liter Tamari.	Basen stickstoff als 100
Purin basen stickstoff	0.215	4.72
Durch Silber nitrat und Baryt		
fällbarer stickstoff	2.686	58.82
Stickstoff in anderer Form	1.655	36.46

Der Hauptanteil der Flüssigkeit diente zur Isolierung der Basen. Zu diesem Zwecke wurde sie mit Salpetersäure neutralisiert und mit 20% Silbernitrat in kleinem Ueberschuss versetzt. In diesem Silbernitrat-Niederschlag sollte man die Purin basen suchen. Die menge desselben reichte jedoch zur weiteren Forschung nicht aus.

#### 1. Der Silbernitrat und Baryt-Niederschlag.

Das Filtrat vom Silbernitrat-Niederschlag wurde mit Silbernitrat und Baryt in mässigem Ueberschuss versetzt. Der dabei entstandene braune Niederschlag wurde nach sorgfältigem Waschen in einem Mörser gebracht und mit Salzsäure verrieben. Man filtrierte nun vom gebildeten Silberchlorid ab und setzte mit Phosphowolframsäure zu. Wird der Phosphowolframsäure-Niederschlag in bekannter Weise mit Baryt zerlegt, so erhält man die Base in freiem Zustande. Die in der Weise dargestellte alkalische Flüssigkeit gab keine Reaktion für Histidin; sie wurde mit Salzsäure neutralisiert, bei gelinder Wärme eingedunstet und im Vakuum-Exikator stehen gelassen. Nach einigen Tage schieden sich die salzsauren Salze der Base als farblose Prismen aus, die aus heissem Wasser umkrystallisiert wurden. Die Ausbeute betrug ungefähr 3 g.

Werden diese Salze mit absolutem Methylalkohol verrieben, so geht der Hauptanteil (etwa  $\frac{2}{3}$ ) in Lösung über während ein Teil ( $\frac{1}{3}$ ) ungelöst bleibt.

A. Der in Methylalkohol unlösliche Teil (*Putrescin*).

Der in Methylalkohol unlösliche Teil betrug etwa 1 g. Aus heissem Wasser unkrystallisiert schied sich das Salz als lange, farblose Prismen aus, die in Wasser leicht, in Aethylalkohol und Aether schwer löslich waren. Im Kapillar rohr erhitzt schmolz es bis 290° nicht. Für die Analyse war das Präparat im Vacuum bei 100° getrocknet.

0.110 g Subst.		0.01941 g N	
0.220 g „		0.09592 g Cl	
		N	Cl
$C_4H_{12}N_2 \cdot 2HCl$	Ber.	17.39	44.09
	Gef.	17.64	43.60

Das Platin chlorid-doppel Salz:--Wird das salzsaure Salz in wenig Wasser gelöst, mit Platin chlorid lösung versetzt und langsam eingedampft, so scheidet sich das Platin-doppel Salz als citronen gelbe unregelmässige Tafeln aus. Es löst sich in kaltem Wasser ziemlich schwer; im Kapillar rohr erhitzt wird es gegen 230° schwarz ohne zu Schäumen. Das gereinigte Präparat war im Vacuum bei 100° getrocknet und analysiert.

0.229 g Subst.		0.0870 g Pt.	
		Pt.	
$C_4H_{12}N_2 \cdot 2HCl \cdot Pt Cl_4$	Ber.	39.20	
	Gef.	39.17	

Das Pikrat:--Zur Darstellung des Pikrats löst man das salzsaure Salz in wenig Wasser, gibt eine entsprechenden Menge Natrium pikrat zu. Das Gemisch wird so lange erwärmt bis klare Lösung auftritt. Nach dem Erkalten scheidet sich das Pikrat als hellgelbe Prismen aus, die in kaltem Wasser schwer löslich sind. Im Kapillar rohr erhitzt wird es gegen 240° dunkel braun und zersetzt sich bei 260° (uncorr.) Für die Analyse wurde es im Vacuum bei 100° getrocknet.

0.2050 g Subst.	0.0412 g N	
		N
$C_4H_{12}N_2(C_6H_3N_3O_7)_2$	Ber.	20.52
	Gef.	20.56

Die Analyse stimmt also mit der Formel  $C_4H_{12}N_2$  oder *Putrescin* überein.

B. Der im Methylalkohol lösliche Teil ( $C_6H_5N_3$ ).

Der in Methylalkohol lösliche Teil betrug ungefähr 2 g. Aus heissem Methyl alkohol ungelöst scheidet sich das Salz als farblose Prismen aus. Im Kapillar rohr erhitzt schmilzt es scharf bei  $232^\circ$  (uncorr.). Für die Analyse war es im Vacuum bei  $100^\circ$  getrocknet.

0.1851 g Subst.	0.03944 g N	
0.1225 g „	0.04471 g Cl	
		N Cl
$C_6H_5N_3 \cdot 2HCl$	Ber.	21.43 36.23
	Gef.	21.31 36.50

Ein Teil des Salzes wurde in bekannter Weise in das Pikrat verwandelt. Es bestand aus citronengelben glänzenden rhombischen Prismen oder Tafeln. Es war in heissem Wasser und Alkohol leicht, in kaltem Wasser schwer und in Aether unlöslich. Im Kapillar rohr erhitzt schmolz es bei  $230^\circ$  (uncorr.) und zersetzte sich unter Schäumen. Für die Analyse war es im Vacuum bei  $100^\circ$  getrocknet.

0.2265 g Subst.	0.04788 g N	
		N
$C_6H_5N_3(C_6H_3N_3O_7)_2$	Ber.	21.69
	Gef.	21.14

Aus der Analyse und anderen Beobachtungen wurde festgestellt, dass diese Base mit der von U. Suzuki u. A. aus Schoyu isolierte Base  $C_6H_5N_3$  identisch war.

II. Das Filtrat vom Silbernitrat und Baryt-Niederschlag.

Das Filtrat vom Silbernitrat und Baryt-Niederschlag wurde nach

dem Entfernen des Silbers durch Salzsäure und des Baryts durch Schwefelsäure, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Die freie Base, die durch Zerlegung des Phosphowolframsäure-Niederschlags durch Baryt in bekannter Weise erhalten war, wurde mit Salzsäure neutralisiert, langsam eingedünstet und im Exikator stehen gelassen. Die salzsauren Salze der Base schieden sich dabei als farblose krystalle aus, die etwa 5 g. betrugen. Diese krystalle liessen sich durch Behandeln mit absolutem Alkohol in zwei Fraktionen trennen.

A. Die in absolutem Alkohol unlösliche Fraktion (*Ornitin*).

Diese Fraktion enthielt eine erhebliche Menge anorganische Salze, die in Methylalkohol unlöslich waren. So wurde sie mit kaltem Methylalkohol sorgfältig verrieben und abfiltriert. Das Filtrat wurde langsam bis zur Trocknen eingedampft und mit absolutem Alkohol und Aether gewaschen. Als der Rückstand aus warmem Wasser umgelöst wurde, schied sich das salzsaure Salz als farblose Prismen aus, die in Wasser leicht, in absolutem Alkohol und Aether schwer löslich waren. Die Ausbeute betrug ungefähr 2 g. Im Kapillarrohr erhitzt schmolz das Salz bei  $203^{\circ}$  (uncorr.) unter Schäumen. Für die Analyse wurde das gereinigte Präparat im Vakuum bei  $100^{\circ}$  getrocknet.

0.2110 g Subst.	0.02924 g N		
0.1463 g „	0.05076 g Cl		
	N	Cl	
$C_5H_{12}N_2O_2 \cdot 2HCl$	Ber.	13.66	34.63
	Gef.	14.00	34.69

Das Pikrat bestand aus glänzenden gelben Tafeln oder sternförmig-verwachsenen Prismen, die in kaltem Wasser schwer, in heissem Wasser dagegen leicht löslich waren. Im Kapillarrohr erhitzt zersetzte es sich bei  $248-250^{\circ}$ . Für die Analyse war es im Vacuum bei  $100^{\circ}$  getrocknet.

0.2120 g Subst.	0.04073 g N		
		N	
$C_5H_{12}N_2O_2 \cdot C_6H_3N_3O_7$	Ber.	19.39	
	Gef.	19.21	

Das Platinchlorid-doppelsalz:—Gelbe Prismen; leicht löslich in Wasser, schwer in Alkohol. Im Kapillarrohr erhitzt zersetzt es sich bei 223°.

0.1620 g Subst.	0.057 g Pt.	
		Pt.
$C_5H_{12}N_2O_2$	$H_2PtCl_6$	Ber. 35.95
		Gef. 35.19

B. In Methylalkohol lösliche Fraktion.

Von dieser Fraktion haben wir nichts isolieren können.

# ZUSAMMENFASSUNG DER RESULTATE.

Aus 1 Liter Tamari wurden isoliert:

Putrescin	0.3 g
Ornitin	0.7
Base $C_6H_9N_3$	0.7
$NH_3$	4.5

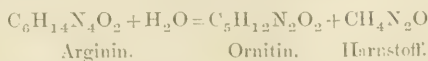
Das Vorhandensein von Arginin, Lysin und Histidin wurde nicht constatiert. Dass das Eiweiss (Glycinin) aus Soja bohnen durch Säure Spaltung ziemlich erhebliche Menge Hexon basen liefert haben T. B. Osborne und S. H. Clapp<sup>1</sup> und auch der Verfasser selbst nachgewiesen. Nach einer quantitativen Analyse wurden die folgenden Zahlen gefunden.

In 100 Teilen Glycinin.

(Osborne und Clapp<sup>1</sup>.) (Yoshimura.)

Arginin	5.12	4.87
Lysin	2.71	2.51
Histidin	1.39	1.85

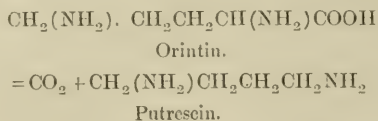
Nach E. Schulze und E. Winterstein<sup>2</sup> wird das Arginin durch Baryt wirkung in Harnstoff und Ornitin gespalten.



1. Amer. Jour. Physiology 1907 19 468—74.

2. E. Schulze u. E. Winterstein: Ber. deutsch. chem. Gesellsch. 1897 30 2879

Das Ornitin soll nach A. Ellinger<sup>1</sup> durch Fäulnis weiter in Putrescin verwandelt werden.



Es ist deshalb höchst wahrscheinlich, dass das Ornithin und Putrescin in Tamari auf Kosten des Arginins gebildet wurden.

Nach U. Suzuki und K. Aso soll die Base  $\text{C}_6\text{H}_9\text{N}_3$ , die auch in gewöhnlicher Schoyu gefunden wurde, wahrscheinlich durch Bakterienwirkung aus Histidin gebildet werden.

Ueber das Schicksal des Lysins muss man die künftige Forschung abwarten.

1. A. Ellinger: Hoppe-seyler, Zeitsch. f. Physiol. Chem. 1902 29 334.



# On the Carbohydrates of Shōyu.

BY

R. Mitsuda.

## 1. Isolation of sugars from shōyu.

Although the existence of sugars in shōyu is easily shown by the reduction of Fehling solution, it is comparatively difficult to isolate them in a pure state. At first, I have tried to isolate them as the lead compounds. For this purpose 400 c.c. of shōyu were evaporated under a low pressure. The separated sodium chloride was removed, the mother liquor was diluted with water and precipitated with basic lead acetate. To the filtrate of the lead precipitate was now added a moderate excess of basic lead acetate and ammonia whereby the greater part of the carbohydrates was thrown down. This precipitate was now suspended in water and decomposed with hydrogen sulphide. The filtrate of lead sulphide which contains the sugars was evaporated in vacuum to expell the hydrogen sulphide and the osazone was prepared in the usual manner.

Afterwards, it was found that such a long operation is unnecessary. After removing the greater part of sodium chloride by evaporation, it was decolorized with animal charcoal and directly warmed with phenylhydrazine solution (for 400 c.c. shōyu were used 25 g. phenylhydrazine hydrochlorate and 33 g. sodium acetate) whereby mixture of various osazones separated out as yellowish crystalline mass. By repeating the recrystallisation of the crude osazones from a hot 60% alcoholic solution, about 5 grs. pure glucosazone were obtained, which was dried in vacuum at 100° and analysed.

0.1674 g Subst.	0.3664 g CO <sub>2</sub>	0.0912 g H <sub>2</sub> O		
0.1263 g „	16.6 c.c. N (10° 765 <sup>mm.</sup> )			
		C	H	N
Glucosazone C <sub>18</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	calc.	60.14	6.14	15.64
	found	59.78	6.05	15.84

Recrystallized from hot water or from 60% alcohol, the glucosazone separates as characteristic yellow needles which melt at  $204^{\circ}$ .

On cooling the filtrate of the glucosazone a little galactosazone was obtained. The crude product was purified at first by washing with cold acetone and then recrystallized from boiling 20% alcohol. In this way I obtained about 0.6 g. pure galactosazone which melts at  $184^{\circ}$ . The determination of nitrogen gave the following result:

0.1326 g Subst.	17.7 c.c. N ( $14^{\circ}$ 766 mm.)
	N
$C_{18}H_{22}N_4O_4$	calculated 15.64
	found 15.75

Although I have recognized the presence of maltosazone under the microscope I was not able to isolate it.

## 2. About furfural.

When shōyu or tamari is exactly neutralized with dilute caustic alkali and subjected to distillation. The distillate produces the red coloration with aniline acetate, which is the characteristic reaction for furfural. So there is no doubt that furfural exists in shōyu or tamari in the free state. But the quantity being too small, it is only possible to determine it by the colorimetric method. For this purpose, a dilute standard solution of furfural was prepared by dissolving 1 g. of pure furfural in 5 liters of water, so that 1 c.c. of the solution contained 0.0002 g. of furfural. For the determination, 100 c.c. of the sample were diluted with water to 120 c.c. exactly neutralized with alkali and subjected to distillation until the distillate gave no more red coloration with aniline acetate. The distillate was then diluted up to 100 c.c.

In two tall beakers of the equal size was put the mixture of 5 c.c. of aniline acetate and 5 c.c. strong hydrochloric acid. To one beaker was added 10 c.c. of the distillate and to the other 10 c.c. water and so much standard furfural solution from a burette, until the same intensity of the red colouration in both beakers was obtained. In this way we

can calculate how many c.c. of the standard furfural solution is equal to 10 c.c. of the distillate. The result was as follows:

No.	Trade mark.	% of furfural.	Total acidity (as butyric acid.)
1	Kikuichi	0.0001	0.28
2	Minakami	0.00015	0.65
3	Homare	0.0002	0.91
4	Tamari No. 1	0.0002	0.91
5	„ No. 2	0.0001	0.29
6	„ No. 3	0.00005	0.26

As the above table shows the amount of furfural is very little in each sample, yet we could find out the differences clearly, because the reaction was very delicate.

We see also in the above table that the amount of furfural is generally greater in those samples which show stronger acidity though there exists no direct proportionality between them.

In the next experiment I found that the amount of furfural increases with the ages of moromi. Thus:

No.	Ages of moromi.	Furfural.	Total acidity.
1	2 weeks	Trace	—
2	1 year	0.0002	0.42
3	2 years	0.0004	0.45
4	3 years	0.0006	0.58

From these facts we can probably conclude that furfural is very gradually produced by the action of organic acid during the ripening process of moromi. This view is further supported by the following fact:

When shōyu or tamari is distilled without neutralization. The furfural reaction in the distillate is far stronger than, when it is previously neutralized. This fact indicates that furfural is formed from pentose or pentosane by the action of acids during distillation. So we can suppose that even at ordinary temperature the formation of furfural is slowly going on during the ripening of moromi.

The view that furfural is produced by microbes during the ripening of moromi, is not probable, because we find no furfural in the newly prepared saké, while the preserved one contains a tolerable quantity of it. In the case of shōyu-moromi the analogy is also found thus; almost no furfural was present in the moromi of two weeks.

Further investigation on this point is desirable.

### 3. About total pentosane.

For the determination of total pentosane 50 c.c. of the sample were mixed with 20 c.c. strong hydrochloric acid and subjected to distillation, adding from time to time a 12% hydrochloric acid solution until the distillate reached 400 c.c. and the furfural in the distillate was precipitated by phloroglucin in the usual way. The result was as follows:

Mask.	Drymatter.	% Pentosane.	% Pentosane in dry matter.
Kikuichi	32.80	0.41	1.26
Homare	46.43	0.50	1.08
Minakami	45.21	0.53	1.17
Tamari No. 1	56.74	0.91	1.18

We see from this result that the quantity of pentosan is directly proportional to the dry matter in shōyu or tamari. We see further that the amount of total pentosane in shōyu and tamari is comparatively very little, while in the original material, in shōyu-koji which is prepared from the same quantity of soja bean and wheat it is contained in far greater quantity. Thus I found in one sample 8.31% of the dry matter. This fact shows that the greater part of pentosane in koji remains insoluble during the ripening process of moromi.

In the next experiment I compared the amount of total pentosane in different stages of ripening of moromi.

Ages of Moromi.	% Pentosane.	% Pentosane in dry matter.
2 weeks	2.03	6.05
1 month	1.60	4.77
5 months	2.17	6.42

1 year	1.89	5.06
2 years	1.98	5.17
3 years	1.79	4.68

The above result shows that the pentosane decreases only very gradually, either by the assimilation of microbes or by the action of organic acids. I found a more striking result when I determined the total pentosane in the pressed juice of moromi at different stages of ripening. Thus:

Age of Moromi.	% Total Pentosane.
2 weeks	0.49
1 month	1.31
5 months	1.37
1 year	0.94
2 years	0.72
3 years	0.67

We see from the above result that the pentose and pentosane in shōyu-koji is gradually dissolved in salt water and reaches the maximum at 5 months and then decreases again gradually.

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# Studies on the Microorganisms of "Tanezu" (Japanese vinegar ferment).

BY

T. Takahashi.

Japanese vinegar is made from various kinds of raw materials:—altered "saké," altered "moromi"-mash of "Saké," vinegar mash<sup>1</sup> especially prepared for this purpose, and "saké-kasu" (pressed residue) of "Saké"-mash. "Kasuzu" is the name generally applied to vinegar prepared from "Saké Kasu."

In preparing "Kasuzu" water and "Saké Kasu," which has been allowed to ferment for about a week, are mixed in certain proportions, and the mash is pressed in a special press. The fluid part thus obtained is divided into two equal parts, one of which is heated to about 80—90°C. This heated fluid is mixed with the other part and after the addition of some previously prepared mash<sup>2</sup> is allowed to ferment for about one month or more.

1. This vinegar mash is prepared by mixing rice "Koji," steamed rice and water, very similar to the "Moromi"-mash in the manufacture of "Sake."

2. This mash is called "Tanezu" in Japanese. Torizō Nishimura\* analyzed "Tanezu" with the following results:—

Sp. grav. (at 12.8°C) .. .. .	1.08
In 100 c.c.	
Volatile substance .. .. .	97.626 grms.
Alcohol .. .. .	5.620 "
Volatile acid (as acetic acid) .. .. .	1.109 "
Extract .. .. .	2.373 "
Total nitrogen .. .. .	0.300 "
Ash .. .. .	0.113 "

\* T. Nishimura: "Kasuzu Jōzōron,"



Thus the addition of a previously fermented mash (*Tanezu*), which contains immense numbers of acetic bacilli, is an essential part in the preparation of vinegar.

It has seemed to the writer that an investigation of the microorganisms of "*Tanezu*" is very important and interesting both from the practical and the scientific points of view.

Two samples were used for the investigation, one<sup>3</sup> from "*Handa-machi*" the other<sup>4</sup> from "*Nagoya*," and 7 different varieties of acetic bacillus, 2 varieties of wild yeast and 2 varieties of *Aspergillus* were isolated. They are described below.

## PART I. BACTERIA.

### No. 1. *Bacterium Ascendans* Henneberg var *Tanezu*.

I.—Form and size: Commonly  $3\mu \times 1\mu$  or  $4\mu \times 1\mu$ . Involution form is found in film of beer culture (one month), but not in "*Sakekasu Sumashi*"<sup>5</sup> even after two months. Chain form was observed in the former culture and each cell was very short appearing almost like a coccus. Nonmotile.

II.—Growth: 1. *Solid culture*: a. *Plate cultures*: - "*Moromi*" agar: Round, elevated, light greyish, waxy colony appears on surface. "*Saké*"-agar: Somewhat bluish-white, waxy, round (almost spherical) colony appears after 7 days at 25°C. Its periphery is waxy when seen with a magnification of 125. b. *Surface cultures*: "*Saké*"-agar: Grows as fine granular covering (40 hours at 30°C), waxy and light rose colored growth of broad stream (24 hours at 35—45°C and further 18 days at

3. A vinegar factory of M. Nakano.

4. A vinegar factory of D. Sasada.

5. A mash made from "*Sakekasu*": 1 kgrms. of "*Sakekasu*" is mixed with 4 l. of water; and after letting stand at the room temperature for about one week, pressed, sterilized, putting in to a tightly corked flask.

30°), or pasty yellow-greyish white covering with almost smooth<sup>6</sup> surface (10 days at 21.2°C or 6 days at 20.5°C). "Koji"-extract-agar: Forms yellow-greyish white pasty coverings (6 days at 20.5). "Koji"-extract-gelatine: Grows very feebly (5 days at 14°C). c. *Stab-cultures*: "Saké"-agar: Forms lightly rose colored pasty growth along the mouth of the stab canal (6 days at 20.5°C), or becomes lustrous at margin (10 days at 25°C). Beer wort-agar: Grows very feebly (6 days at 20.5°C). "Koji"-extract-agar: Forms dirty yellow, pasty, finely granulated growth at the mouth of the stab canal (6 days at 20.5°C). "Koji"-extract-gelatine: Forms creamy growth along the stab canal (38 days at 16.5°C).

2. *Fluid culture*: (6 days at 20.5°C).

<i>Culture media</i> I.	Remarks.
Yeast water: Forms island on surface. Fluid clear. No sediment.	
Yeast water glucose:	Do.
Wort <sup>7</sup> (without hop): Forms ringshaped growth. Fluid clear.	
Wort: Somewhat thick film <sup>8</sup> was formed on surface. Fluid clear and without sediment.	
"Koji"-extract: Forms somewhat thick dirty yellow film. Fluid clear and almost no sediment.	
Hyduck's solution: Growth almost nil.	
Bouillon: Forms ring-shaped growth.	

Thus we can distinguish this bacillus from No. 2, No. 3, No. 4, No. 5, by the formation of rather thick film on the surface of the medium. Further, its inability to avail itself of asparagin as a nitrogen source (Hayduck's solution) and the circumstance that the medium remains clear are characteristics which distinguish it from the already known varieties of *B. ascendans*.

6. *Bacterium aceti*, Brown, var *Tanezu* forms under the same conditions (10 days at 21.2°C) very lightly rose colored colony with granular surface.

7. The wort used in this investigation was prepared from malt (70%) and rice (30%).

8. Thicker than *B. aceti*, Brown, var *Tanezu*.

*Culture media II.*

## Remarks.

"Saké Kasu"-mash: Forms very thin (perfectly smooth) film (9 days at 25.6°C), but sediment is comparatively conspicuous. Fluid becomes turbid after shaking. Film becomes blue when treated with solution of I+KI.

Beer<sup>9</sup>: Forms very thin film on the wall of the vessel. Fluid clear (7 days 25°C), but when shaken, the film became agglutinated into one mass. Total acidity increase by 0.9912% after one month.

Wine<sup>10</sup>: No growth after 20 days (at 30°C), but after 5 days more a cohesive film appeared.

Pasteur's solution<sup>11</sup>: Forms very thin film after 6 days at 30°, fluid remaining clear.

Beijerinck's solution<sup>12</sup>: No growth (7 days at 25°C).

"Saké"<sup>13</sup>: No growth (30 days at 25°C).

Diluted "Saké" (water 20%): No growth (30 days at 25°C).

Diluted "Saké" (water 30%): Do.

Diluted "Saké" (water 50%): Forms film on the wall of the vessel, the medium remaining clear. Folds were formed more or less after three months.

9. and 10. Beer and wine used was "Sapporo" beer and "Kikusui shirushi" natural wine.

9. "Sapporo" beer: It contained 4.62% of alcohol and 0.094% of total acidity as succinic acid.

10. "Kikusui Shirushi" natural wine, containing 3.86% of alcohol and 0.67% of total acidity.

11. Glacial acetic acid	12.5 c.c.	Absolute alcohol	22.5 c.c.
Ammonium phosphate	...	...	0.2 gr.
Potassium	...	...	0.1 gr.
Calcium	...	...	0.1 gr.
Magnesium	...	...	0.1 gr.
Water	...	...	1000 c.c.
12. Water	100 c.c.	Alcohol	3 c.c.
Ammonium phosphate	...	...	0.05 gr.
Potassium	...	...	0.01 gr.
13. Alcohol	17.6%	Total acidity. (as succinic acid)	0.1711%
Volatile acid	0.024%	Extractive matters	3.457%
Sugar	0.965%		

Thus we see that this bacillus can assimilate ammonium nitrogen, and it must therefore be a variety of *B. aceti*. Pasteur according to Hoyer's<sup>14</sup> classification of acetic bacillus.

III.—Behavior towards carbohydrates and alcohol. The production of acid from carbohydrates was tested with yeast-water or bouillon<sup>15</sup> containing a carbohydrate (at 25°C). The results are given below:—

Substance.	Growth.	Acid production.	
		<i>B. ascendans</i> Henn. Var Tanezu	<i>B. ascendans</i> Henneberg.
Glucose.	A thin ring formed after 8 days. Fluid clear, not becoming turbid on shaking.	+++	—
Fructose.	Forms very thin film after 8 days. Fluid slightly turbid and the film sinks on shaking.	++	—
Galactose.	Forms very thin ring after 8 days. Fluid remained clear, but became turbid on shaking.	—	—
Rhamnose.	Turns very slightly turbid after 3 days. Forms white and brittle film, which breaks on shaking, the fluid remaining clear notwithstanding.	—	/
Saccharose.	Forms islands and ring after 7 days, fluid clear. After 8 days more Fehlings solution gave no reaction.	+	—
Maltose.	Forms white film along wall of test tube, fluid clear.	—	—
Arabinose.	Forms trace of ring and film after 8 days. Film sinks to bottom on shaking	++	—

14. Deutsche Essigindustrie 1899. Nr. 1.

15. These media were used for all the varieties,

Lactose.	Forms trace of ring and slight turbidity after 7 days.	trace	—
Raffinose.	Do.	—	—
Mannitol.	Forms very thin and slight turbidity after 3-18 days.	—	—
Dextrin. <sup>16</sup>	Forms very thin film and a little turbidity after 3 days. Fluid became clear after 15 days more.	—	—
Starch.	Forms white film and a little turbidity after 7 days.	—	—
Inulin.	No growth after 3 days, but after 15 days more a white brittle film was formed.	—	/
Ethylalcohol.		+++	+

Thus this bacillus forms acid from arabinose, glucose, fructose, saccharose, lactose and dextrin and hereby distinguished from the *B. ascendans* Henneberg already known.

IV.—Fermentation products: Propyl; ethyl-, methylalcohol and fuseloil were found in the distillate of koji-extract culture after 4 days at 25—26°C. but acetic<sup>17</sup> and butyric acids, acetone, and methylacetate were not found in it.

V.—Conditions of temperature: Optimum temperature lies above 30°C. Grows very slowly below 14°C, but very energetically above 35°C and produces a rose color. 55°C for 10 minutes in "Saké Kasu"-mash<sup>18</sup> does not kill the cell, which dies at 80°C.

From the above description we see that this bacillus belongs to Hoyer's quick vinegar bacilli or *B. aceti* Pasteur and must be looked upon as a variety of *B. ascendans* Henneberg.

16. This substance contained more or less glucose.

17. This acid was found in the culture of diluted "Saké" or "Saké"—agar.

18. This mash was used for this purpose throughout these studies.

No. 2. *Bacterium Accetosum* Henneberg var *Tanezu*.

I.—Form and size: Commonly  $2.5 \times 1\mu$  or  $3\mu \times 1\mu$  and diplococcus or often long chain. Involution form is found in film of beer culture (one month); .....Non-motile.

II.—Growths: 1. *Solid medium*: a. *Plate cultures*: "Moromi"-agar: Round greyish-yellow colony with fine concentric rings and radiations. Central part of colony somewhat elevated but margin rather flat with wavy<sup>19</sup> periphery. "Saké"-agar: Forms somewhat elevated light yellow, waxy colony, with a special elevation in the central part. Under the microscope the periphery is very irregular. b. *Surface cultures*: "Saké"-agar: Forms dirty greyish white covering with coarse granulated surface, and very thick folded film<sup>20</sup> on surface of condensed water (24 hours at  $35^{\circ}$ — $45^{\circ}\text{C}$  and 2 days more at  $30^{\circ}\text{C}$ ). After 18 days at  $30^{\circ}\text{C}$ , the surface growth changes to milky. Forms slightly folded covering and very small film on condensed water after 3 days at  $22$ — $25^{\circ}\text{C}$ ), or grows waxy in streaks, on which *watery drops* form after 4 days at  $23.7$ — $24^{\circ}\text{C}$ , but after the lapse of 2 months the culture surface turns wet and granular. "Koji"-extract-agar (at  $23.7$ — $24^{\circ}\text{C}$ , 4 days): Grows in white and obscure streaks (thick part of medium) or semi-transparent (rather thin part of medium) and granular in middle but slant and smooth in periphery. Surface layer generally wet. "Koji"-extract-gelatine: Weak growth in streaks (4 days at  $14^{\circ}\text{C}$ ), or yellowish creamy growth (38 days at  $16.5^{\circ}\text{C}$ ).

In general this bacillus grows better on "Saké"-agar than on "Koji"-extract agar.

c. *Stab cultures*: "Saké"-agar. Forms white and folded colony at mouth of stab canal (4 days at  $23.7$ — $24^{\circ}\text{C}$ ). Wort-agar: On mouth forms white pasty colony. "Saké"-gelatine: Trace of growth at mouth (4 days at  $14$ — $15^{\circ}\text{C}$ ). "Koji"-extract-gelatine: Weaker growth than on "Saké"-gelatine (4 days at  $14$ — $15^{\circ}\text{C}$ ). Neutral "Koji"-extract-gelatine: very weak growth.

19. The wavy contour is observable from the back side of the colony.

20. At  $26$ — $29^{\circ}\text{C}$  for the same length of time, there was no film.

2. Fluid medium: (4 days at 23.7—24°C).

*Culture media I.*

Remarks.

Yeast water: Forms film. Fluid clear, with sediment.

Yeast water-glucose: Do.

Wort (without hop): Forms slimy film. Fluid clear with more or less sedimentation.

Wort: Forms ring after 3 days at 25°C, after further 4 days appeared film accompanying turbidity.

"Koji"-extract: Forms somewhat thick film with some deposits under clear fluid.

Hayduck's solution: Forms thin brittle film with some deposits under clear fluid.

Bouillon: Forms thin film with turbid deposits.

The turbidity of the bouillon culture and the formation of a slimy film in wort are points of similarity between this bacillus and *B. aceti* Hansen. It can be distinguished from bacillus No. 1, by the thickness of the film.

*Culture media II.*

Remarks.

"Saké-kasu"-mash: Forms white folded film<sup>21</sup> after 4 days (25.6°C), the fluid becoming turbid. Growth exceedingly rapid, paralleled only by No. 7. (*B. Xylinoides*). Sediment becomes rose red in color after very long culture.

Beer: Forms trace of deposit after 7 days (at 25°C), and thin film over clear fluid after 9 days. Total acidity increased by 1.3334% after one month.

Wine: No growth even after 30 days (at 30°C).

Pasteur's solution: No growth at 30°C (after 26 days) and hereby distinguishable from *B. aceti*, *Pastorianus*.

Beijerinck's solution: No growth after 7 days at 25°C.

Diluted "Saké" (water 20%): No growth even after one month at 25°C.

<sup>21</sup>. This film stains yellow with I+KI solution.



Diluted "Saké" (water 30%): Forms trace of sediment after 25 days (25—28.5°C.).

Diluted "Saké" (water 50%): Forms very thin film with trace of ring over very turbid fluid after 25 days at 25—28.5°C. Film thickens after 3 months and forms folds and a part of it extend upwards along wall of apparatus.

### III.—Behavior towards carbohydrates and alcohol.

Substance.	Remarks.	Acid production.	
		B. acetosum. Henn. Var Tanezu	B. acetosum. Henneberg.
Arabinose.	Forms film over turbid fluid after 8 days at 25°C. Film settles down after breaking on shaking	+++	—
Glucose.	No growth after 5 days at 25°C. Turbidity commence on surface part after 11 days.	+++	+
Fructose.	Forms film with a trace of turbidity after 8 days at 25°C.	—	—
Galactose.	No growth after 8 days at 25°C.	/	+
Rhamnose.	No growth after 3 days at 25°C, but after 15 days more forms somewhat thick film with production of turbidity. Film does not break on shaking	—	—
Saccharose.	Forms white film <sup>22</sup> after 7 days (at 25°C), it settles down without breaking on shaking, and therefore causes almost no turbidity.	trace	—

22. Under the same condition bacillus: No. 1. (B. ascendans, Henneberg, var Tanezu) forms islands and rings, but no film.

Maltose.	Forms white and folded film, <sup>23</sup> after 8 days at 25-26°C which settles down without breaking and causing almost no turbidity.	—	—
Lactose.	Forms white film <sup>24</sup> after 7 days at 25°C, with a trace of turbidity. Film breaks on shaking, causing turbidity.	—	—
Raffinose.	Almost no growth even after 17 days. <sup>25</sup>	+	—
Mannitol.	No growth after 3 days, but after 15 days more forms very thin film a part of which settles on shaking.	—	—
Dextrin.	No growth after 3 days at 25°C, but after 15 days more became slightly turbid.	++	—
Starch.	Forms white <sup>26</sup> ring with slight turbidity after 7 days at 25°C, and after 2 days more forms white easily broken film.	—	—
Inulin.	No growth after 3 days at 25°C, but after 15 days more forms film a part of which breaks on shaking, causing turbidity. <sup>27</sup>	—	—
Ethylalcohol.		+++	+

23. Bacillus No. 1. does not form film under the same condition.

24. Bacillus No. 1. forms a ring under the same conditions.

25. Unlike, bacillus No. 1. which forms a ring with some turbidity after 7 days.

26. Bacillus No. 1. forms film under the same condition.

27. Bacillus No. 1. does not cause turbidity.

Thus, this bacillus forms acid from arabinose, saccharose, raffinose, dextrin and hereby distinguished from the *B. acetosum* Henneberg already known.

IV.—*Fermentation products.* Propyl, ethyl, methyl-alcohol and fuseloil were found in the distillate of Koji-extract culture after 4 days at 25-26°C, but acetic and butyric acids and methyl-lactate were not found in it.

V. Conditions of temperature: Optimum temperature for growth lies above 30°C. Grows very slightly below 14°C. Heating to 55°C for 10 minutes in "saké-kasu" mash does not kill the cell, which dies at 80°C.

This bacillus belongs to Hoyer's beer vinegar bacilli, *Bact. rancens* Beijerinck, and has many similarities to *B. acetosum* Henneberg. Further, it is distinguished from *B. acetii* Brown by its property of forming reducing sugar from mannitol.

No. 3. *Bacterium acetii* Brown var *Tanezu* I.

I.—Form and size: Short bacillus commonly  $2\mu \times 1\mu$ ,  $3\mu \times 1\mu$ . Involution form is found in film of beer culture (one month), or "Saké-kasu"-mash (2 months). Nonmotile.

II.—Growth: 1. *Solid medium*: a. *Plate culture*: "Maromi"-agar: Round elevated greyish white, moist colony appear on surface. Round colony with smooth margin in interior of medium (at 125/I) "Saké"-agar: Forms round elevated greyish white, moist colony. b. *Surface culture*: "Saké"-agar Forms greyish yellow, pasty, smooth, lustrous covering at 30°C after 40 hours, or dirty yellow, pasty, lustrous covering with clear condensed water after 24 hours at 35-45°C and 2 days more at 30°C. Covering becomes rose color after 18 days at 30°C, and greyish white at 25°C after 18 days. Covering of old culture slimy<sup>28</sup>.

"Koji-extract"-agar (3 days at 25°C): Forms semi-transparent covering on surface of thin part of medium, while white pasty on thicker

28. Another variety described in this paper does not form slimy covering.

part. Forms film on clear condensed water. "Koji-extract"-gelatine: Forms dirty yellow creamy growth (38 days at 16.5°C).

C. Stab-culture: "Saké-agar": Forms greyish white pasty growths along mouth of stab canal (4 days at 23.7-24°C). Wort-agar: Forms lustrous pasty growths over mouth of stab canal. (4 days at 23.7-24°C). "Koji-extract"-gelatine: Makes pasty pin head like growths at mouth of stab canal. (4 days at 14-15°C). "Saké"-gelatine: Same as in "Koji-extract"-gelatine.

## 2. Fluid media (4 day, at 23.7-24°C).

### *Culture media I.*

### Remarks.

Yeast water: Forms islands on surface. Fluid clear, with some deposits.

Yeast water glucose: Forms islands on surface. Fluid clear and no sediment.

Wort (no hop): Forms lusterless film and ring with sedimentary growths along wall of test tube. (after 7 days at 25°C).

Wort: Forms white ring, but no film and sediment.

"Koji"-extract: Forms film and sediment.

Hayduck's solution: Forms white easily broken ring.

Bouillon: No growths after 4 days, but after 6 days more there appeared a thick ring and much sediment.

Thus, the film of this bacillus is easily to break without causing turbidity.<sup>29</sup>

### *Culture media II.*

### Remarks.

"Sakékasu"-mash: Film begins to form after 5 days at 25-26°C and after 4 days more dense turbidity comes with somewhat rose red colored sediments. Film stains yellow with I+KI solution. The turbidity formed in this medium was characteristic of this bacillus, and not observed in the other 6 varieties. Film became very thick after 48 days more at 16°C.

29. The culture on "Sakékasu"-mash is an exception on this point.

Beer: Forms somewhat thick film, which settles down on breaking and forms very thin film and ring (after 6 days at 25°C). Total acidity increased by 6.75% (of which 5.904% was acetic acid).

Wine: No growths after 20 days at 30°C.

Pasteur's solution: No growths after 26 days at 30°C, hereby distinguishable from *B. aceti* Pasteur.

Beijerinck's solution: No growths after 7 days at 25°C.

"Saké": No growth after one month at 25°C.

Diluted "Saké" (water 20%): No growth after one month at 25°C.

„ (water 30%): Do.

„ (water 50%): Forms ring with turbidity and much sediment after 25 days at 25-28.5°C. Forms thin film over turbid fluid after 3 months more.

### III.—Behavior towards carbohydrates and alcohol:

Substance.	Growth.	Acid production	
		Bact. aceti. Brown. var Tanezu.	Bact. aceti.
Arabinose.	A trace of sediment after 12 days at 25°C.	++	—
Glucose.	Do.	+	+
Fructose.	Do.	++	—
Galactose.	Almost no growths.	+(?)	+
Rhamnose.	Forms white easily broken film after 2 days at 25°C. Film does not settle down on shaking.	—	—
Saccharose.	No growths after 7 days at 25-26°C but after 8 days more there was formed a thin film and ring, which caused turbidity on shaking.	—	—
Maltose.	No growths after 8 days at 25-26°C, but after further 3 days more a ring was formed, which caused turbidity on shaking.	--	—

Lactose.	No growths after 15 days at 25°C.	—	—
Raffinose.	No growths after 7 days at 22.5°C, but after 9 days more a thin and easily broken film was formed, which caused turbidity on shaking.	—	—
Mannitol.	Forms thin film after 2 days at 25°C. On shaking it caused a trace of turbidity. Fluid reduced Fehling's solution very distinctly after 22 days culture.	—	—
Dextrin.	Forms thin film after two days at 25°C and a part of it formed flocculent particles in fluid.	++	—
Starch.	Forms a trace of turbidity after 7 days at 22.5°C.	+	—
Inulin.	Forms ring and flocculent particles in fluid after 20 days at 25°C.	—	—
Ethylalcohol.		+++	—

The property of forming acid from arabinose, fructose, dextrin, and starch is a distinguishing feature of this variety which differentiates it from the *Bacterium aceti* already known. Further, its inability to assimilate lactose, arabinose, glucose, fructose, and galactose distinguishes it from the other 6 varieties.

IV. Fermentation products: The growth was very poor in "Koji-extract" in Erlenmeyer's flask and therefore the products were not examined.

V. Conditions of temperature: Optimum temperature for growth lies near 30°C, growth very slow below 14°C. Heating to 55°C for 10 minutes does not kill the cell, which dies at 80°C.

Thus, this variety belongs to *Bact. rancens* Beijerinck (Hoyer's system) and it has the character of forming reducing sugar from mannitol in common with *Bact. aceti* Brown.

No. 4. *Bacterium aceti* Brown var *Tanezu* II.

I.—Form and size: Short bacillus. Cells forming ring in "Saké-kasu"-mash culture (2 months) are irregular in size:—long ones 5  $\mu$ , short ones 2-3  $\mu$  long, and 1.5—2  $\mu$  broad generally. Chain form occurs very often, and each cell of the chain is very short appearing almost like a coccus. Involution forms in beer or "Saké-kasu"-mash are very irregular (see plate):— $l=15\mu$   $b=5.2\mu$  Non-motile.

II.—Growth: 1. *Solid medium*: a. *Plate culture*: "Moromi"-agar (7 days at 25°C): Forms round elevated light greyish-white lustrous colony on surface. Marginal part has bud-like unevenness. (125/I). "Saké"-agar: Round elevated dirty yellow, waxy colony appears on surface. (3 days at 21.7°C). Marginal part waxy (125/I). Rose red color developed on colonies after a very long time.

b. *Surface culture*: "Saké"-agar: Forms dirty white, filmy covering (40 hours at 30°C) or lustrous dirty-white pasty growths and smooth film on condensed water (24 hours at 35—45°C and 20 days more at 30°C). On thicker part of medium forms mesenteric folds. (18 days at 30°C or 18 days at 26°C). Rose red coloration of covering strongly developed above 30°C and weakly below 26°C. Forms brown growths after 4 days at 23.7—24°C. "Koji-extract"-agar: Forms non-lustrous waxy covering, middle part of the track is granular, its two sides are nearly smooth, and external sides are folded. (4 days at 23.7—24°C). "Koji-extract"-gelatine: No growth after 5 days at 14°C, but after 38 days at 16.5°C transparent colonies are formed along the tracks.

c. *Slab Culture*: "Saké"-agar: Trace of growth. Beer-wort-agar: Forms pasty weak growths at mouth of canal (5 days 25°C). "Koji-extract"-gelatine: Trace of growth (5 days at 15°C). "Saké"-gelatine: Trace of growth (5 days at 15°C).

2. *Fluid media*: (4 days at 23.7-24°C).

*Culture media* I.

Remarks.

Yeast water: Forms islands, with some turbidity and sediment.

Yeast water glucose: Film thicker<sup>30</sup> and turbidity greater<sup>31</sup> than in the above medium.

Wort (not hopped): Forms a trace of ring (7 days at 25°C) over clear fluid.

Wort: Forms thin film.

"Koji-extract": Forms a trace of island.

Hayduck's solution: No growth, but sediment formed after 10 days more.

Bouillon: Forms thin film with more or less turbidity and sediment.

Thus the property of forming an easily broken film and the turbidity of the nutrient fluid are characteristic of this bacillus and distinguished it from the 3 varieties above described. *Bact. ascendans* always forms turbidity in the nutrient fluid, but in this bacillus its formation depends upon the kind of medium used.

#### *Culture media II.*

#### Remarks.

"Saké Kasu" mash: Forms very thin film after 5 days at 25.6°C, and after 4 days more it becomes somewhat rose colored and forms many folds, but the fluid remains clear<sup>32</sup>. Film stains yellow with 1 + KI. solution.

Beer: Forms very thin<sup>33</sup> film growing upwards along side of apparatus after 7 days at 25°C. Fluid below film is very turbid, as in *Bact. ascendans*. Film breaks on shaking. After one month total acidity increased by 5.056% (of which 4.926% was acetic acid).

Wine: No growth after 20 days at 30°C.

Pasteur's solution: No growth after 26 days at 30°C, and hereby distinguishable from *Bact. aceti* Pasteur.

Beijerinck's solution: No growth after 7 days at 25°C.

"Saké": No growth after one month at 25°C.

Diluted "Saké" (water 20%): No growth after one month at 25°C.

Diluted "Saké" (water 30%): Do.

30. and 31. This feature was not observed in No. 1. and No. 2.

32. Same as in No. 1. and No. 2. but different from No. 3.

33. Thinner than the film of No. 1. bacillus.



Diluted "Saké" (water 50%): Forms non-lustrous spotted film,<sup>34</sup> over clear fluid after 25 days at 25-28.5°C.

III.—Behavior towards carbohydrate and alcohol:

Substance.	Growth.	Acid production.		
		Bact. acti Brown, var Tanezu. II.	B. acti Brown. var Tanezu. I.	Bact. oxidans.
Arabinose.	No growths after 8 days at 25°C, but after 4 days more forms very thin film, which breaks easily on shaking and causes turbidity.	+++	++	+
Glucose.	Forms some turbidity with a trace of ring <sup>35</sup> formation, after 8 days at 25°C.	+++ ++	+	+
Fructose.	Forms a trace of ring with little turbidity and sediment. (8 days at 25°C).	+(?)	—	+
Galactose.	Forms a trace of ring with little turbidity after 8 days at 25°C. Ring breaks on shaking and causes turbidity.			
Rhamnose.	Forms very thin film and some turbidity after 2 days at 25°C.	--	—	—
Saccharose.	Forms no film but turbidity after 7 days at 25°C.	—	—	+
Maltose.	Forms a trace of ring with little turbidity, very similar to No. 6. bacillus described further on, after 8 days at 25—26°C.	++	—	+
Lactose.	Forms no film but trace of ring, with turbidity of fluid after 7 days at 25°C. After 8 days more			

34. The film was thicker than in No. 3.

35. This ring does not break on shaking

	began to form film.	—	No growth.	+
Raffinose.	Forms very thin film with very strong turbidity after 7 days at 22.5—26°C.	—	—	—
Mannitol.	Forms very thin film, which easily breaks on shaking, causing turbidity. Fluid reduces Fehling's solution very well.	—	—	—
Dextrin.	Forms very thin film over clear fluid after 2 days at 25°C. After 19 days more fluid was shaken but fragments of film adhered to wall without causing turbidity.	+	++	+
Starch.	Forms no film but trace of turbidity after 7 days at 25°C.	+	+	—
Inulin.	No alteration after 10 days more. No growth after 2 days at 25°C, but after 19 days more the fluid turned turbid with formation of easily broken film.	—	—	—
Ethylalcohol.		++++	++++	+

The property of this bacillus to form acid from galactose, saccharose, lactose, raffinose, mannitol and starch distinguishes it from the *Bact. oxidans* already known, and that of producing reducing sugar from mannitol assimilates it to *Bact. aceti* Brown.

IV. Fermentation products: Ethyl and methylalcohol and fusel oil were found in the distillate of the culture of "Koji-extract," but isopropyl alcohol, acetone, methylacetate, isobutyl-alcohol, acetic-acid and butyric acid were not found in it.

V. Conditions of temperature: Optimum temperature for growth lies near 30°C, the growth is very much retarded at 26°C, and almost entirely inhibited below 14°C. Heating to 55°C for 10 minutes will not kill the cell which dies at 80°C.

According to Hoyer's system, this bacillus belongs to *Bact. rancens* Beijerinck, and the property of producing reducing sugar from mannitol assimilates it to *Bact. aceti* Brown, but the formation of rose red color at high temperatures is characteristic of this bacillus.

No. 5. *Bacterium acetosum* Henneberg var *Tanezu*.

I.—Form and size: Short bacillus,  $1\mu \times 0.5\mu$  or  $3\mu \times 1\mu$  commonly isolated or forming chains; Involution form appears very frequently in film of "Saké-kasu"-mash (2 months culture), and measures  $38\mu \times 1\mu$  or  $50\mu \times 2.5\mu$ . In film on beer, involution form appears very seldom and many of them form chains.

II.—Growth: 1. *Solid medium*: a. *Plate culture* "Moromi"-agar: Forms round colony becoming rose red in color in old culture. "Saké"-agar: Forms round elevated light greyish creamy colony on surface, but in deeper part it appears like lens and the margin is uneven with bud-like growths. (125 L).

b. *Surface culture*: "Saké"-agar: Forms dirty white mesenteric covering after 40 hours at  $30^{\circ}\text{C}$ , or rose red sharply folded covering like that of mycoderma yeast, also folded film on condensed water. (24 hours at 35-45 and further 2 days at  $30^{\circ}\text{C}$ ), or no film on condensed water but with intense rose covering (18 days at  $30^{\circ}\text{C}$ ).<sup>36</sup> Still another culture forms yellowish-white covering of which the thicker part began to become rosy. "Koji-extract"-agar: Forms semitransparent covering. (6 days at  $20.6^{\circ}\text{C}$ ). "Koji-extract"-gelatine: No growth after 5 days at  $14^{\circ}\text{C}$ , but forms yellowish creamy growth after 38 days at  $16.5^{\circ}\text{C}$ .

c. *Stab culture*: "Saké"-agar: Forms rose red pin-head-like growth with granular surface at mouth of stab-canal. (after 6 days at  $20.5^{\circ}\text{C}$ ). Wort-agar. Forms some what rose red pin-head-like growth at mouth of stab-canal. (6 days at  $20.5^{\circ}\text{C}$ ). "Koji-extract"-gelatine: Forms trace of growth at mouth of stab-canal (6 days at  $17.19^{\circ}\text{C}$ ). "Saké"-gelatine: Forms growth at mouth of stab-canal. (6 days at  $17.19^{\circ}\text{C}$ ).

36. In the same culture at  $26^{\circ}\text{C}$  there was production of the rose red color but the mass of covering was smaller than (almost half) at  $30^{\circ}\text{C}$ .

## 2. Fluid media. (6 days at 20.5°C).

*Culture media I.*

## Remarks.

Yeast water: Forms islands over clear fluid with little sediments.

Yeast water-glucose: Forms islands over clear fluid with little sediments.

Wort (not hopped): Forms islands over clear fluid.

Wort:

Do.

"Koji"-extract: Forms very thin film, but thicker than in any other culture of this bacillus.

Hayduck's solution: No growth.

Bouillon: Forms trace of film over turbid and sediment holding fluid.

Like bacillus No. 1 (*B. ascendans* Henneberg), this bacillus can not assimilate asparagin, and in bouillon alone turbidity was observed. The latter property was also observed bacillus No. 2. (*Bact. acetosum* Henneberg).<sup>37</sup>

*Culture media II.*

## Remarks.

"Saké-kasu"-mash: No growth after 4 days (at 25.6°C), but after 5 days growth begins and after 10 days a thick, folded,<sup>38</sup> dirty yellow film is formed. Film stains yellow with I + KI solution.

Beer: Forms thin film which grows upwards along wall of apparatus, but fluid remains clear with little sediment. (after 7 days at 25°C). After one month total acidity increased by 2.525%.

Wine: (7 days at 30°C): Forms more or less thick film, which partly grows upwards along wall of apparatus. Film sinks down as one mass, on shaking:—a characteristic of *B. acetosum*.

Pasteur's solution: No growth after 26 days at 30°C, hereby distinguishable from *B. aceti* Pasteur.

Beijerinck's solution: No growth after 7 days at 25°C.

37. But *Bact. acetosum* Henn. forms only a film in Hayduck's solution, a point of difference from this bacillus.

38. The formation of such folded film was also observed in *B. aceti*. Brown, var. *Tanezu* II, but in this case it was rose colored.

"Saké": No growth even after one month at 25°C.

Diluted "Saké" (water 20%): No growth even after one month at 25°C.

Diluted "Saké" (water 30%): Do.

Diluted "Saké" (water 50%): Forms film growing upwards along wall of apparatus after 25 days at 25-28.5°C.

Of the 7 varieties described in the present paper, this variety alone grows in wine.<sup>39</sup>

### III.—Behavior towards carbohydrates and alcohol:

Substance.	Growth.	Acid production.		
		B. acetosum. Henn. var. Tanezu. II.	B. acetosum. Henn. var. acetosum Tanezu. I.	Dact. Henn.
Arabinose.	Forms trace of film after 4 days at 25°C, and after 3 days more forms ring with some turbidity.	trace.	+++	—
Glucose.	Begins to form film and ring after 4 days at 25°C. Ring breaks on shaking, causing turbidity.	++	++++	+
Fructose.	Forms thin film after 4 days at 25°C, and after 3 days more forms ring, which breaks on shaking and causes turbidity.	trace.	—	—
Galactose.	Forms thin film and trace of ring after 4 days at 25°C, and after 3 days more fluid below film became turbid.	—	/	—
Rhamnose.	Forms very thin film, but fluid is clear after 2 days at			

39. Acetic bacilli reported lately these bulletin Vol. VI. No. 4.) behave differently towards wine i.e. varieties  $\alpha$  and  $\delta$  form ring,  $\eta$  forms thin film,  $\beta$  and  $\gamma$  make no growth after 20 days at 30°C.

	25°C. After 19 days more a slight turbidity became apparent.	—	—	—
Saccharose.	Forms islands over intensely turbid fluid after 7 days at 22.5—26°C, but no inversion of sugar took place.	+	trace.	—
Maltose.	Forms no film and turbidity, but trace of sediment after 7 days at 25—26°C, and after 3 days more ring formed, which breaking easily on shaking.	+	—	—
Lactose.	Forms no film, but intense turbidity after 7 days at 25°C, and after 7 days more forms very thin film.	—	—	—
Raffinose.	No growth after 17 days at 22.5—26°C.	?	+	/
Mannital.	Forms thin film over turbid fluid after 2 days at 25°C, after 9 days more appearance remains same, but fluid reduces Fehling's solution.	—	—	—
Dextrin.	Forms very thin film after 2 days at 25°C.	++	++	—
Starch.	No growth after 17 days at 25°C.	?	—	—
Inulin.	Forms very thin film after 2 days at 25°C, after 19 days more film remains almost unchanged	—	—	—
Ethyl alcohol.		+++	+++	+++

The property of this bacillus of forming acid from fructose, maltose, and starch distinguishes it from *Bact. acetosum* Heinberg var. *Tanezu* I. and that of forming reducing sugar from mannitol is common to it and of *Bact. aceti* Brown. Further, the quicker growth of this bacillus as compared with the above described 4 varieties in nutrient media containing arabinose, glucose, fructose, galactose is to be noted.

IV.—Fermentation products: Traces of ethyl-alcohol, fusel-oil, acetone, methyl-lactate and butyric acid were found in the distillate of "Koji-extract" culture of this bacillus, but isopropyl-, methyl-alcohol isobutylalcohol and acetic-acid were not found.

V.—Conditions of temperature: Optimum temperature for growth lies about 30-35°C; growth retarded at 26°C and very slow at 14°C. Heating to 55°C for 10 minutes does not kill the cell which dies at 80°C.

By the character of the film, this bacillus would be referred to *Bact. acetosum* Heinberg, *Bact. rancees* Beijerinck of Hoyer's system, but its property of forming reducing sugar from mannitol assimilates it to *Bact. aceti* Brown.

No. 6. *Bacterium aceti* Pasteur var. *Tanezu*.

I.—Form and size: Very short bacillus commonly  $2\mu \times 1\mu$ . Involution form was not found both in "Saké-kasu"-mash culture (2 months) and in beer culture (one month). Chain form appears very frequently in beer culture. 2 cells united appear in "Saké"-agar surface culture. (22 days at 25°C),  $2.5\mu$  long, but rarely  $5\mu$  long. Non-motile.

II.—Growth: 1. *Solid media*: a. *Plate cultures*: "Maromi"-agar: Round, somewhat yellowish creamy colony appears on surface. "Saké"-agar: Round, somewhat bluish-yellow creamy colony appears on surface. Appears like 2 crossed discs in deeper part of medium (7 days at 20-21.5°C).

b. *Surface culture*: "Saké"-agar: Forms transparent and lustrous covering (40 hours at 30°C), or transparent and bright covering and film over condensed water with sediment (24 hours at 35-45°C and

2 days more at 30°C), but after 16 days more the sediment became rose red. Color production was almost same at 30°C (18 days) and 26°C (18 days), thus differing from No. 4 and No. 5. (Bact. acetii Brown. and Bact. acetosum Hennch.).

Further, there was no difference in growth at 30°C and 22.5-25°C. Forms semitransparent smooth growth and film on condensed water after 7 days at 25°C. "Koji-extract"-gelatine: Forms a trace of semitransparent covering after 5 days at 14°C, or yellowish moist creamy growth after 38 days at 16.5°C.

"Moromi"-agar: Forms semitransparent filmy growth and film on condensed water. (7 days at 25°C).

c. *Stab-culture*: "Saké"-agar: Forms small colony at mouth of canal. (7 days at 25°C).

*Wort-agar*: A trace of growth (7 days at 25°C). "Saké"-gelatine: Forms semitransparent small colony at mouth of stab-canal (6 days at 17-19°C).

## 2. Fluid media: (7 days at 25°C).

### *Culture media I.*

#### Remarks.

Yeast water: Forms no film but a trace of turbidity.

Yeast water glucose: Forms no film but a trace of turbidity.

Wort (no hop): Forms semitransparent ring, over clear fluid.

Wort: Forms semitransparent ring, over clear fluid.

"Koji"-extract: Begins to form ring, over clear fluid.

Hayduck's solution: No growth.

Bouillon: Forms trace of ring.

Thus, turbidity is observed only in yeast water culture, and no growth takes place in Hayduck's solution, as in bacillus No. 1. and No. 5., but the semi-transparent ring is characteristic of the bacillus.

### *Culture media II.*

#### Remarks.

"Saké-kasu"-mash. No growth after 4 days at 25.6°C but after 6 days more there was formed a very thin film, which caused no



turbidity on shaking. After 48 hours more at 16°C film altered into thick leather<sup>40</sup>-like substance.

Beer: Ring began to be formed after 7 days at 25°C, after 2 days more, fragment of film settled down with little turbidity of fluid, and after 22 days more thin and easily broken film formed again. After 34 days total acidity increased by 2.8792%.

Wine: No growth after 20 days at 30°C.

Pasteur's solution: Forms no film and turbidity but sediments after 21 days at 30°C, a characteristic of *Bact. aceti*.

Beijerinck's solution: No growth after 7 days at 35°C.

"Saké": No growth after one month at 25°C.

Diluted "Saké" (water 20%): No growth after one month at 25°C.

Diluted "Saké" (water 30%): Do.

Diluted "Saké" (water 50%): Forms greyish easily broken film after 3 months at 25°C.

### III.—Behavior towards carbohydrates and alcohol.

Substance.	Growth.	Acid production.	
		<i>Bact. aceti</i> , <i>Pasteur</i> , var. <i>Tanezu</i> .	<i>Bact.</i> , <i>acetigenum</i> .
Arabinose.	Forms trace of turbidity after 8 days, at 25°C.	+++	—
Glucose.	Forms trace of ring, fluid remains clear, no turbidity on shaking. (4—8 days at 25°C).	+	+
Fructose.	Trace of turbidity (8 days at 25°C).	+++	—
Galactose.	Forms ring which does not break on shaking. (7 days at 25°C).	+	—
Rhamnose.	No growth after 2 days (25°C), but after 19 days more there was formed ring and white flocculent mass, which caused turbidity on shaking.	—	—

40. This occurred also with bacillus No. 3 and No. 7.

Saccharose.	Trace of turbidity after 7 days (at 22.5—26°C), but after 8 days more film formed and turbidity increased. Sugar was inverted.	++++	—
Maltose.	Begins to form ring and turbidity after 8 days (25—26°C), but after 16 days more white flocculent mass was formed in fluid.	+	—
Lactose.	Forms no film but trace of turbidity after 7 days (at 25°C) but after 11 days more formed ring.	+	—
Raffinose.	No film, but intense turbidity, after 8 days ring formed.	trace	—
Mannitol.	No growth after 2 days (25°C), but after 19 days more ring formed and turbidity appeared.	+	+
Dextrin.	No growth after 2 days (25°C), but after 19 days more ring formed, turbidity and flocculent mass appeared.	trace	—
Starch.	Forms no film but trace of turbidity after 7 days (25°C) but after 7 days more increased turbidity and after 4 days more ring commence to form	++	—
Inulin.	No growth after 2 days (25°C), but after 19 days more ring formed and turbidity appeared.	--	—
Ethylalcohol.		+++	+

By its property of bringing forth turbidity in all the media except galactose yeast water, this bacillus differs from all the above described varieties and *Bact. acetigenum* and *Bact. aceti* Pasteur. Its property of forming acid from sugars differentiates it clearly from *Bact. acetigenum*

and *Bact. ascendans*, but the power of inverting cane sugar is also possessed by *Bact. aceti*. The film of the galactose yeast water culture stains *reddish-brown*, and that of the glucose-yeast-water *dark blue* with the cellulose reagent ( $I + H_2SO_4$ ), and a similar reaction is known to occur especially with the film of *Bact. xylinum*.

IV.—Fermentation products: Traces of methyl-alcohol and fusel-oil were found in the distillate of "Koji"-extract culture, but no ethyl-, isopropyl-alcohol, methyl-lactate, acetone, acetic acid or butyric acid.

V.—Conditions of temperature: Optimum temperature for growth lies near  $22-30^{\circ}C$ , minimum at  $14^{\circ}C$ . Heating to  $55^{\circ}C$  for 10 minutes does not kill the cells.

This bacillus belongs to *Bact. aceti* Pasteur of Hoyer's system, but differs from *B. acetigenum* and *B. ascendans* in many respects.

No. 7. *Bacterium xylinoides* var *Tanezu*.

I.—Form and size: Long bacillus. Involution form was not found in film on "Saké-kasu"-mash (2 months culture), size  $5\mu \times 1\mu$  or  $6\mu \times 1\mu$ . In growth of "Saké"-agar surface culture, the cell measures:  $7.5\mu \times 1.25\mu$  or more commonly  $2.5\mu - 3\mu \times 1\mu$ . The cells are usually isolated but combinations of two occur very rarely. Non-motile.

II.—Growth: 1. *Solid media*: a. *Plate cultures*: "Maromi"-agar: Forms yellowish white creamy colony. Marginal part irregular with bud-like outgrowths (125/I).

"Saké"-agar: Forms brown spherical colony. (7 days at  $20-21^{\circ}C$ ).

b. *Surface cultures*: "Saké"-agar: Forms dirty white, smooth, non-lustrous pasty growth (40 hours at  $30^{\circ}C$ ), or non-lustrous dirty white filmy covering (1 day at  $35-45^{\circ}C$  and 2 days at  $30^{\circ}C$ ), after 16 days more growth becomes dirty brown<sup>11</sup> and forms smooth film on condensed water. Forms dirty white pasty growth and film on condensed water with much sediments (8 days at  $22-25^{\circ}C$ ), which becomes rose red after 19 days more. Film, becomes yellowish-brown after 40

<sup>11</sup>. The Brown color is deeper in cultures made at  $26^{\circ}C$  than at  $30^{\circ}C$ .

days. Makes better growth at 22-25°C than at 30°C. Forms dirty yellowish brown covering with stream on margin. (7 days at 25°C). "Maromi"-agar: Forms dirty yellow filmy covering and somewhat thick film on condensed water. "Koji"-extract-gelatine: No growth. (5 days at 14°C).

c. *Slab-cultures*: "*Sake*"-agar: Forms filmy elevated (at central part) growth at mouth of canal, the elevated part is colored rose red (7 days at 25°C). "*Moromi*"-agar: Form dirty yellow flat and smooth colony at mouth of canal. (7 days at 25°C). *Wort*-agar: Forms small dirty brown colony on mouth of canal. (7 days at 25°C). "Koji"-extract-agar: Same as on *Wort*-agar.

## 2. Fluid media. (7 days at 25°C).

### *Culture media I.*

### Remarks.

Yeast water: Forms mouldy growth remaining suspended in the medium.

Yeast water glucose. Forms mouldy growth remaining suspended in the medium.

Wort (no hop): Forms film on clear fluid.

Wort: Forms very thin film, which does not cause turbidity on shaking.

"Koji"-extract: Same as in yeast water.

Hayduck's solution: Forms no film but dense turbidity and mouldy suspended growth.

Bouillon: No growth after 40 days (25°C).

Thus, the mouldy growth formed by this bacillus in many nutrient media distinguishes it from the other 6 varieties, but it has the power of assimilating amido-nitrogen in common with *Bact. xylinum*.

### *Culture media II.*

### Remarks.

"Saké-kasu"-mash: Forms film after 4 days (25.6°C), after 6 days more, marginal part of film alters to brown, and after 18 days more film thickens, half leatherly half slimy, and forms thread when treated with platinum wire. Stains very light yellow with 1+KI solution.

Beer: Forms trace of sediment (7 days at 25°C), after one month more total acidity increased by 0.0118%.

Wine: No growth. (20 days at 30°C).

Pasteur's solution: No growth. (21 days at 30°C).

Beijerinck's solution: Do.

"Saké": No growth (one month at 25°C).

Diluted "Saké" (water 20%). No growth (3 months at 25°C).

Diluted "Saké" (water 50%). Do.

Diluted "Saké" (water 50%). Do.

### III.—Behavior towards carbohydrates and alcohol.

Substance.	Growth	Acid production.
		<i>B. Xylinoides</i> var <i>Tanezu</i> .
Arabinose.	Forms ascending film after 8 days at 25°C.	+++
Glucose.	Almost no growth.	?+
Fructose.	Forms transparent film and mouldy growth below, which settles down cause turbidity on shaking.	++
Galactose.	Forms islands, which do not cause turbidity on shaking.	+
Rhamnose.	Forms trace of turbidity. (2 days at 25°C).	--
Saccharose.	Forms mouldy and Semitransparent film, a part of which settles down on shaking, and after 8 days more film thickens. Inversion of sugar was distinctive.	+++
Maltose.	Same growth as in saccharose.	++
Lactose.	Forms no growth after 7 days (25°C) but after 4 days more forms mouldy growth.	trace.
Raffinose.	Same growth as in saccharose.	+++
Mannitol.	Forms no growths after 2 days (25°C), but after 18 days more formed mouldy growth.	trace.

Dextrin.	Forms ring and intense turbidity after 20 days at 25°C.	++
Starch.	Forms mouldy film after 15 days at 22°5—26°C.	trace.
Inulin.	Forms mouldy film after 20 days (25°C).	+
Ethylalcohol.		+++

The property of causing turbidity in rhamnose- and dextrin-yeast-water culture, of the formation of mouldy growth in all the other media, and the inversion of cane-sugar are also found in *Bact. xylinum* already known.

Further, treatment with the cellulose reagent (Jodine + Potassium iodid + concent  $\text{H}_2\text{SO}_4$ ) of these films gave the following result:—

Media, film formed.	Color produced.
Fructose yeast water.	Reddish brown.
Saccharose " "	Brown.
Maltose " "	Brown.
Raffinose " "	Light yellow.

The colors produced are thus not those peculiar to cellulose, so this bacillus must be referred to *Bact. xylinoides*.

IV.—Fermentation products. Isopropyl-(trace), ethyl and methyl-alcohol, fusel-oil, methylactate were found in the distillate of "Koji-extract" culture, but not acetic acid, acetone, or butyric acid.

V.—Conditions of temperature: Optimum temperature for growth lies near 22-25°C. At 30°C or above, the growth is rather retarded. Heating to 55°C for 10 minutes kills the cell.

This bacillus belongs to *Bact. xylinoides* of Henneberg, the film of which gives the cellulose reaction, which this variety does not, as far as my experience goes.

## PART II. YEAST AND MOULDS.

Besides bacteria, certain kinds of mycodermia and *Torula* were found.

The former grow in "Koji"-extract but emit no odor; the latter also flourish in "Koji"-extract but hardly in "Saké-kasu"-mash. Therefore, these two varieties of yeast probably do not play an important part in "Kasuzu" (Sake-kasu-vinegar) manufacture. Among the moulds, *Aspergillus oryza* and *Asp. glaucus* were isolated from Nakano's sample, and a variety of *Aspergillus* forming intensely green spores was also found in Sasada's.

### SUMMARY.

1. The majority of the micro-organisms of "Tanezu" which playing an important role during the manufacture of "Kasuzu" (a kind of Japanese vinegar), are bacteria, which may belong to.

1. *Bact. raneens*.
2. *Bact. acetii* Pasteur.
3. *Bact. xylinoides*.

and may be subdivided into 7 varieties.

2. The involution from of these 7 varieties was not always present and the production of the rose red color is an interesting character of these cultures. All varieties grow in diluted "Saké" (water 50%), except No. 7.

3. The amount of acid produced is variable according to the varieties: some 5% others 1%.

4. The fermentation products in *alcohol free* media differ according to the varieties:—some forms methyl-alcohol and fusel-oil, others form isopropyl-alcohol, ethyl-alcohol, methyl-alcohol and fusel-oil and one, methyl-lactate or butyric acid.

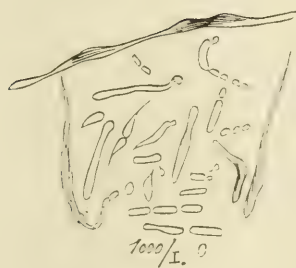
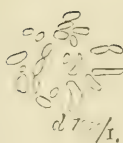
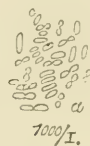
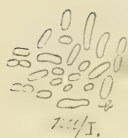
NOTE:—*Bacillus* No. 1.—No. 5, were isolated from Nakano's sample, *Bacillus* No. 6. and No. 7, from Sasada's. In Nakano's sample *Bacillus* No. 2, predominated, and in Sasada's No. 6.

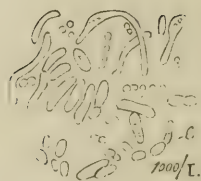
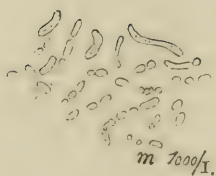
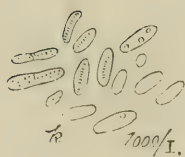
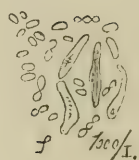
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## EXPLANATION OF PLATE.

- a—k. The cells of beer-agar culture (23 days).  
l—m. The cells of film of beer-culture (one month).  
e and g. 750/L, a—d, f, h—m 1000/L.
- a. No. 6. *Bact. aceti* Pasteur var Tanezu.  
b. No. 7. *Bact. xylinoides* var Tanezu.  
c. No. 4. *Bact. aceti* Brown Tanezu II.  
d. No. 6. *Bact. aceti* Pasteur var Tanezu.  
e. No. 5. *Bact. acetosum* Henneberg var Tanezu II.  
f. No. 3. *Bact. aceti* Brown var Tanezu I.  
g. No. 4. *Bact. aceti* Brown var Tanezu II.  
h. No. 2. *Bact. acetosum* Henneberg var Tanezu I.  
k. No. 7. *Bact. xylinoides* var Tanezu.  
l. No. 1. *Bact. ascendans* Henneberg var Tanezu.  
m. No. 2. *Bact. acetosum* Henneberg var Tanezu I.
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# A Preliminary Note on the Varieties of *Aspergillus Oryzae*.

BY

T. Takahashi.

Since *Asp. Oryzae* is the fungus which plays an important rôle in the brewing of Japanese Saké, various authors have studied this fungus, but thus far no attention has been paid to the existence of its varieties.

The writer has isolated 3 varieties from 3 kinds of "Tanekoji"<sup>1</sup> from three different sources<sup>2</sup>.

1. Variety with very long air-mycelium (Luft mycel) found in Tanaka's sample. Spores are formed very late.

2. Variety with short air-mycelium found in Ueda's sample.

3. Variety with short air-mycelium found in Higuchi's sample.

1. Morphology: On Koji-extract-agar the following morphological characters were observed:

	I.	II.	III.
Breadth of mycelium.	2.5—3.0 $\mu$	2.5—3 $\mu$	2.5—3 $\mu$ .
Length of conidiophore.	1.0 c.m.	1.5—2 m.m.	1.5—2.0 m.m.
Diameter of the "Head."	40.—60 $\mu$	70—90 $\mu$	60—100 $\mu$ .
Diameter of the "Blase."	25—35 "	50—60 "	30—65 "
Diameter of the conidiophore.	10—17.5 "	7.5—17.5 "	75—9 "
Thickness of the wall of conidiophore.	—	1—1.5 "	—
Diameter of the sterigma.	2.5 "	2.5 "	2.5 "
Diameter of the conidia.	5—10 "	6—7.5 "	6—7 "

1. The word "tane" means "seed," therefore Tane-koji relates to the spores of *Aspergillus Oryzae*.

2. K. Tanaka at Kyoto; Yanesuke Ueda at Ōsaka and M. Higuchi at Ōsaka.

The lengths of the conidiophores on koji-extract gelatine (at 10-18°C after 30 days) were:—

I.	II.	III.
1.0—1.5 c.m.	3—5 m.m.	1—2 m.m.

Further, the lengths of the same on Hayduck's solution were:—

I.	II.	III.
2—3 c.m.	0.5—0.8 c.m.	0.3—0.5 c.m.

In regard to morphological characters, the breadth of the "Blasen" and the length of the conidiophore have to be considered as differential characters of the varieties.

II. Physiological differences: On koji-extract-agar plate culture at 25-32°C, there appears, after 4 months, in var. I a white mycelium and bluish-brown spores together with a few brown spores, in var. II dark spores, and in var. III brown spores.

On koji-extract-gelatine at 10-18°C after 30 days: in var. I bright yellow spores or bluish-yellow spores with liquefaction of gelatine; in var. II bluish-yellow spores and but little liquefaction; in var. III, bright yellow spores with only a trace of liquefaction.

The same culture after 40 days: All three varieties had brown spores; after three months more, the gelatine was liquefied and crystals of *ca-oxalate*<sup>3</sup> appeared in I and III, but none in var. II.

In Hayduck's solution at 25-23°C after 16 days; in var. I very light yellow spores appeared but the solution remained uncolored; in II and III bluish-yellow spores and yellow solution. In glycerin<sup>4</sup> Hayduck's solution after 26 days at 28-32°C: in I dark brownish spores, in II light brown spores, in III brown spores and yellow solution. After 34 days more the solution became yellow also in II, and after 51 days<sup>5</sup> a very light yellow

3. The formation of this substance by fung has long been known, especially in *Asper. niger* and *Penicillium glaucum*, but its formation by *Asper. Oryzae* was quite recently observed by K. Saitō, who isolated this fungus from *soya-koji*.

4. Glycerin was used instead of sugar in Hayduck's solution.

5. That is 77 days from the beginning.

color of appeared in the solution in I. This *polychrome* property was observed already by Siebermann, Wehmer, and recently by Milburn and by K. Saitō.

On boiled rice at 27-28°C after 7-10 days: dark yellow brown spores appear in I, and light yellow spores in II and III; at 31-32°C, after 4-5 days greenish-blue spores in II and III, no spores in I even after 14 days<sup>6</sup>. Thus, the first variety fructifies well only at a rather low temperature, but the other two varieties do so also at higher temperatures.

*Enzyme production:* The Extracts of the cultures of the three varieties grown on boiled rice were prepared with 5% alcohol and to 3 c.c. of these extracts was added 3 c.c. of starch paste (2.5%) and the mixture kept at 60-65°C. After 50 minutes no starch reaction was obtained in the case of II and III, and a moderate one with I.

Further, tests for oxidase and peroxidase showed their absence in these extracts, while catalase was present in all the three. The extracts all turned dark after some time in the presence of chloroform. Some more fresh extract was precipitated by adding a large quantity of absolute alcohol. The precipitate was collected on a filter and after washing with ether dissolved in water. This solution was tested separately with tyrosin and hydroquinone; a dark coloration soon appeared with hydroquinone in the case of II and III but not with I, proving that the oxydising enzyme in question was absent in I. *Tyrosinase*<sup>7</sup> was absent in all the three varieties.

On heating the three extracts or the solutions of the alcoholic precipitates to 50°C for 90 seconds, the darkening power was lost, while *catalase* remained active even at 52-53°C, showing that the oxidising enzyme is different from catalase. Further, no H<sub>2</sub>S was produced from sulphur by these extracts. When the extracts were made after the formation of the spores, the amount of oxidising enzyme was evidently much less.

Summary: There exist evidently three varieties of *Aspergillus*

6. A few spores were formed after 21 days.

7. Also phloroglucin was not changed.

Oryzae, differing in their morphological and physiological properties; i.e. length of the conidiophore, color production in the nutrient fluid, optimum temperature for spore formation, speed of liquefying gelatine, and presence of an *oxidising enzyme*.

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## Ueber den Einfluss der höheren Temperatur beim Sterilisieren der Milch.

VON

Y. Kida.

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Bekanntlich ist Soxhlet gegen Anwendung höherer Temperatur beim Sterilisieren der Milch und zwar deshalb, weil dadurch die Milch eine unerwünschte Färbung annimmt und die Verdaulichkeit auch dadurch abnimmt.

Bendix<sup>1</sup> hat auch dieselbe Meinung geäußert.

Wir beobachten, dass der Geschmack und die Farbe der Milch durch Sterilisation sich verändert. Das Aroma der frischen Milch geht verloren; sie nimmt den bekannten etwas bitteren Geschmack an und die weisse Farbe ändert sich, wohl im Folge einer teilweisen Caramelisierung des Milchzuckers, in eine gelbliche bis bräunliche Färbung um.

Die Geschmacksveränderung tritt, wie Duclaux behauptet, schon bei 70° plötzlich ein.

Da indessen Geruchs- und Geschmacksempfindungen beim Säuglinge und bei jungen Kindern noch sehr wenig entwickelt sind, so kommen diese Veränderungen der Milch wohl kaum in Betracht. Wichtiger ist die Veränderung der Verdaulichkeit und Ausnutzbarkeit der Eiweissstoffe und des Fettes, die die Milch beim Sterilisieren erleidet. Wenn man die Milch, die zuvor über 100° oder auf 100° erhitzt worden ist, längere Zeit ruhig stehen lässt, so bildet sich eine starke Fettschicht, der Rahm, auf der Oberfläche der Milch, der sich durch heftiges Schütteln nur schwer auseinander reissen lässt und dann in grosse Klumpen zerfällt. Dagegen bildet sich bei frischer oder aufgekochter Milch, beim Stehen, ein lockerer Rahm, der sich durch Schütteln wieder in feine Partikelchen in der Milch verteilt.

Diese mit blossem Auge wahrnehmbare Veränderung, d.h. die Umwandlung des Milch fettes aus dem Zustande der feinen Emulsion in den der groberen Klumpen bildung, muss natürlich der Resorption des Fettes nachtheilig sein.

Trotzdem giebt es noch einige Forscher, die als besondere Vorzüge der steriliserten Milch vor der rohen, neben der Keimfreiheit und Haltbarkeit, die leichte Verdaulichkeit und bessere Verwerthbarkeit für Säuglingen und Kinder hervorheben.

Also die Frage "Rohe oder gekochte Milch?" bleibt immer noch unentschieden. Es war nun der Hauptzweck des Verfassers, die oben erwähnten Tatsache nochmals zu prüfen und experimentell festzustellen, ob die gekochte Milch wirklich minderwertiger als die frische ist. Darüber hatte der Verfasser auf folgende zwei Punkte seine Aufmerksamkeit gerichtet, viz.

1. Die Veränderung der Verdaulichkeit der Eiweissstoffe.
2. Die Veränderung des Lecithin gehalten beim Sterilisieren.

Um den Verdauungsgrad der Eiweiss stoffe zu bestimmen, wurde es in folgender Weise ausgeführt.

Man bereitet zuerst eine Verdauungs flüssigkeit, indem man 1g Pepsin in einem Erlenmeyer kolben in 500c.c. 0.2% iger Salzsäure löst; hierzu giebt man 20g der zu untersuchenden Milch probe zu und lässt es bei einer Temperatur von  $37-40^{\circ}$  20-24 Stunden unter öfterem Umschütteln stehen. Nach der Versuchszeit werden die nicht verdauten Eiweissstoffe (d.h. durch Pepsin nicht angegriffene Eiweissstoffe; hauptsächlich Casein), in der Milch in bekannter weise bestimmt. Es wurde gefunden:

Die nicht verdauten Eiweissstoffe  
in 100 g Milch

Nicht erwärmt	0.762 g
30 Minuten auf $80^{\circ}$ erwärmt	1.153 g
" $85^{\circ}$ "	1.493 g
" $90^{\circ}$ "	1.420 g
" $95^{\circ}$ "	1.540 g
" $100^{\circ}$ "	1.719 g

30 Minuten im Autoklaven (3 Atm. Druck) erhitzt



Eiweiss gehalt in der Vollmilch	3.462 g
Die verdauten Eiweissstoffe in % der	Die verdauten Eiweissstoffe in der
Gesamt-Eiweissstoffe	nicht erwärmten Milch als 100
78.0	100
66.7	85.5
55.9	72.9
59.0	75.6
56.1	71.9
55.5	71.2
50.4	64.6

Am obigen Resultate sieht man, dass die Verdaulichkeit der Eiweissstoffe in der erhitzten Milch bedeutend abgenommen hat.

Es fehlt auch an Tierversuche nicht, die konstatieren, dass die Eiweissstoffe und Fette in der frischen Milch besser ausgenutzt werden als in der gekochten.

2. Die Bestimmung des Lecithingehaltes der Milch wurde in folgender Weise ausgeführt.

1 Liter Milch wurde zu gewisser Temperatur erwärmt und dann wurde sie bei niederem Druck verdampft. Der Rückstand wurde mit wenig trockenem Gyps vermischt, vorsichtig verrieben. Dieselbe wurde nun mit Aether und dann zweimal je 2 Stunden mit kochendem Alkohol extrahiert. Die beiden Auszüge wurden verdampft. Der Rückstand wurde mit Soda und Salpeter geglüht, in verdünnter Salpetersäure gelöst und die Phosphorsäure bestimmung in gewöhnlicher Weise nach Molybdän methode ausgeführt. Der gefundene Phosphor wurde zum Lecithin umgerechnet. Es wurde gefunden.

	KUHMLICH (I)	
	Nicht erwärmt	30 minuten auf 95° erwärmt
Lecithin in 1000cc.	0.467 g	0.349
Abnahme		0.118
% ige Abnahme		25.27%
	KUHMLICH (II)	
	Nicht erwärmt	30 minuten auf 80° erwärmt
Lecithin in 1000cc.	0.505	0.467
Abnahme		0.038
% ige Abnahme		7.52%

## KUHMITCH (III)

	Nicht erwärmt	30 minuten auf 80°	30 minuten auf 75°
Lecithin	0.474	0.420	0.444
Abnahme		0.054	0.030
% ige Abnahme		11.39%	6.33%

## KUHMITCH (IV)

	Nicht erwärmt	30 minuten auf 100°	30 minuten im Autoclaven über 100°
Lecithin	0.351	0.351	0.407
Abnahme		0.111	0.116
% ige Abnahme		21.22%	22.17%

Bei diesem Resultate sieht man die deutliche Abnahme des Lecithin-gehaltes bei der stark erhitzten Milch.

Bordas und Sig. de Raczowsky<sup>2</sup> nehmen an, dass die Verdauungs- und Ernährungsstörung bei Neugeborenen, welche ausschliesslich mit erhitzter Milch genährt werden, wenigstens zum Teil auf die Verminderung des Lecithins zurückzuführen ist.

Bei der leichten Pasteurisierung bleiben die wertvollen Fermente ungestört, die Eiweissstoffe zeigen nur wenig Veränderung. Der Geschmack ändert sich nicht und der Lecithingehalt nimmt nicht ab. Derartige Milch wird nicht nur gern genommen, sondern ihre Verdaulichkeit steht kaum hinter der rohen zurück und ausserdem die pathogenen Keime werden unschädlich gemacht.

So ist das Pasteurisieren der Milch bei niederen Temperaturen auszuführen.

2. Zeitschrift. f. Nahrungs-und Genussmittel. 1091. B. VII. S. 91.

## Researches on the Preservation of Night-Soil.

BY

K. Asō and S. Nishimura.

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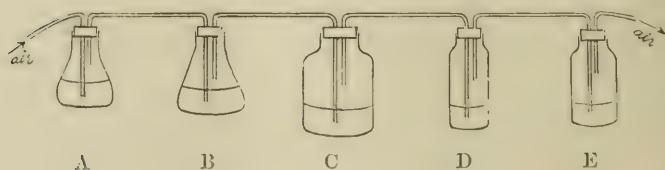
Night-soil forms the principal manure used by the farmers of Japan. It contains all fertilizing ingredients, but of greatest importance are nitrogenous compounds which consist chiefly of more or less decomposed proteins. It is well known that night-soil lose a part of its nitrogen during the storage with liberation of ammonia and free nitrogen, and various methods for preventing, or rather of diminishing the loss of that nitrogen have been proposed, without, however, reaching the desirable degree of perfection. In this regard also three communications have been published in Japan, viz. by the Agricultural College in Tōkyō, by the former Sanin-branch-station of the Imperial Agricultural Experimental Station and by the chemical laboratory of the Central Experimental Station in Tōkyō. These experiments led to the conclusion that the loss of nitrogen from night-soil may be diminished during the storage by well closing the storage-vessels, and by the addition of superphosphate as had been also recommended by others. The addition of superphosphate and gypsum had the purpose to transform the carbonate of ammonia into unvolatile ammonium compound. In a similar way kainit may serve to fix ammonia on account of its containing magnesium sulphate. According to the investigation of the Central Imperial Experimental Station, the addition of superphosphate to night-soil diminished very considerably the loss of nitrogen, especially the volatilization of ammonia as seen from the following figure .

	Loss of Nitrogen.		Loss of Ammonia.	
	Control.	Superphosphate added.	Control.	Superphosphate added.
After 1 month.	100	96.8	100	25.2
" 2 "	100	92.1	100	26.3
" 3 "	100	82.0	100	19.8
" 4 "	100	71.0	100	19.1
" 5 "	100	48.6	100	18.3

It appeared to me of considerable interest to compare the effect of the superphosphate with that of gypsum and kainit, since the former, on account of its acid reaction, might have merely prevented the desirable rotting process of the night-soil.

#### EXPERIMENT IN THE LABORATORY.

The apparatus used for this experiment was composed of a series of connecting bottles as seen from the following drawing:



The flask C contained the sample of night-soil; A and E contained strong sulphuric acid in order to retain ammonia from the air to be passed through the apparatus. D contained a normal solution of sulphuric acid to absorb ammonia volatilized from the sample and B contained water to supply air saturated with moisture to the sample. A definite volume of air was passed through this apparatus, every day. The night-soil was passed through a sieve, then after well mixing with an equal volume of

water divided into six portions, each of 195 g., and mixed with various preserving compounds.

I.—Superphosphate, 5% of the sample.

II.—Gypsum which contained  $\text{SO}_3$  in an equivalent quantity to that of the superphosphate added.<sup>1</sup>

III.—Chemically pure monocalcium phosphate in an equivalent quantity to that of superphosphate.<sup>2</sup>

IV.—Pure calcium sulphate and calcium phosphate, in the same quantities as in the second and in the third bottles.

V.—Kainit which contained  $\text{SO}_3$  in an equivalent quantity to that of gypsum in superphosphate added.

VI.—Night-soil alone.

The superphosphate used in this experiment contained 15.30%  $\text{P}_2\text{O}_5$ , soluble in water and 20.45%  $\text{SO}_3$ , and the kainit 12.0%  $\text{SO}_3$ . The temperature during this experiment was:

Maximum.	Minimum.	Average.
13.°5C.	4.°8C.	9.°3C.

After three weeks, the putrefied samples were analysed with the following result:

	Total nitrogen.
Original.	0.725 %
I.	0.614 %
II.	0.608 %
III.	0.619 %
IV.	0.609 %
V.	0.608 %
VI.	0.513 %

From these figures the loss of nitrogen during the storage was calculated as follows:

1. The total content of  $\text{SO}_3$  in the superphosphate was determined although there was certain difference between the total  $\text{SO}_3$  and  $\text{SO}_3$  in gypsum contained in the superphosphate.

2. In this case also total  $\text{P}_2\text{O}_5$  soluble in water was assumed to be equal to that in the form of monocalcium phosphate.

	Loss of nitrogen.
I.	0.111 %
II.	0.117 %
III.	0.106 %
IV.	0.116 %
V.	0.117 %
VI.	0.212 %

The quantity of ammonia collected in the bottle D, was as follows:

	Ammoniacal nitrogen volatilized.
I.	0.068 %
II.	0.088 %
III.	0.064 %
IV.	0.059 %
V.	0.069 %
VI.	0.103 %

These results show that superphosphate is very effective agent to diminish the loss of nitrogen and to fix ammonia, and its power depends, chiefly upon its content of monocalcium phosphate, and not to its admixture of gypsum.

In regard to the loss of nitrogen in the free state further experiments were necessary.

### EXPERIMENT IN THE FIELD.

This experiment was conducted to determine the change of various nitrogenous organic compounds in night-soil during the time of the usual storage in the field.

On May 17, samples well prepared according to the method above mentioned, were placed into six porcelain jars<sup>3</sup>, each containing 3,500 grams of the sample. Each jar was put in the field soil, the upper rim being a few inches higher than the surface of the ground, and all were arranged in the same condition in a space enclosed with straw, only one side of this space being opened. The reagents added and the ratios

3. Generally Japanese farmers use wooden tubs or concreted tubs for this purpose.

of their quantities used were quite similar to those of the preceeding experiment. The samples were stirred from time to time and after eighteen days analysed with the following results:

## ORIGINAL SAMPLE.

	%	Total quantity in 3500 g.
Total Nitrogen ... ..	0.989	34.608 g.
Ammoniacal Nitrogen ... ..	0.337	11.795 g.
Organic basic Nitrogen... ..	0.170	6.258 g.
Monoamido Nitrogen ... ..	0.088	3.097 g.
Albuminoid Nitrogen ... ..	0.385	13.458 g.

	Loss of weight. g.	Remaining weight. g.
I.	705.0	2795.0
II.	700.7	2799.3
III.	364.2	3135.8
IV.	545.1	2954.9
V.	598.4	2901.6
VI.	700.0	2800.0

## TOTAL NITROGEN.

	%	Quantity of N. remained, g.	Original. = 100
I.	1.087	30.373	87.70
II.	0.965	27.005	78.03
III.	1.104	34.613	100.01
IV.	1.054	31.148	90.00
V.	0.936	27.177	78.50
VI.	0.909	25.455	70.77

From this result, it becomes evident that the action of monoceleimn phosphate is very pronounced and that gypsum and kainit were not so effective as commonly considered.

		I.	II.	III.	IV.	V.	VI.
Ammoniacal Nitrogen.	{ %	0.452	0.406	0.450	0.435	0.328	0.358
	{ Total quantity g.	12.636	11.351	14.102	12.860	9.523	10.024
Organic basic Nitrogen.	{ %	0.0438	0.1487	0.1303	0.1698	0.2088	0.1799
	{ Total quantity g.	1.224	4.163	4.086	5.017	6.058	5.037
Monoamido Nitrogen.	{ %	0.185	0.062	0.088	0.106	0.077	0.118
	{ Total quantity g.	5.182	1.744	2.762	3.147	2.240	3.296
Albuminoid Nitrogen.	{ %	0.405	0.348	0.436	0.343	0.322	0.254
	{ Total quantity g.	11.331	9.747	13.663	10.123	9.355	7.008

These results show that superphosphate and monocalcium phosphate<sup>4</sup> prevent the loss of nitrogen and fix ammonia more energetically than gypsum and kainit<sup>5</sup>, while they diminish also the decomposition of albuminoids more than those. Gypsum and kainit have but little efficacy in preventing loss of nitrogen.

### ANALYTICAL METHODS.

To determine ammoniacal nitrogen, the sample was diluted with water, mixed with about 2 grams of magnesia usta, and distilled at low temperature and low pressure (40°, 40 mm.). The distillate was introduced into the solution of normal titrated sulphuric acid and titrated back with standard soda solution.

To determine organic basic nitrogen, about 20 grams of the sample was mixed with a concentrated solution of basic lead acetate to precipitate albuminoids, and to the filtrate obtained after well washing the residue, dilute sulphuric acid was added in a slight excess to remove the excess of lead acetate, filtered and washed. The filtrate therefrom was mixed with so much concentrated sulphuric acid that the resulting fluid contained about 5% of sulphuric acid and 10% solution of phosphotungstic acid was added to this mixture. The precipitate was allowed to deposit for 24 hours, and after well washing with 5% sulphuric acid, the residue was subjected to Kjeldahl's method and the difference between the quantity of nitrogen and that of ammoniacal nitrogen found before, was taken as the nitrogen of organic bases.

The determination of albuminoid nitrogen was carried out according to Stutzer's method, and that of total nitrogen, according to Kjeldahl's method.

4. As monocalcium phosphate was chemically pure, a proportionally larger amount was applied than the real content of that in superphosphate.

5. It is probable that the double decomposition between ammonium carbonate and metallic sulphates might be not easily caused in the ordinary case, but according to the law of mass-action this reaction might be explained.

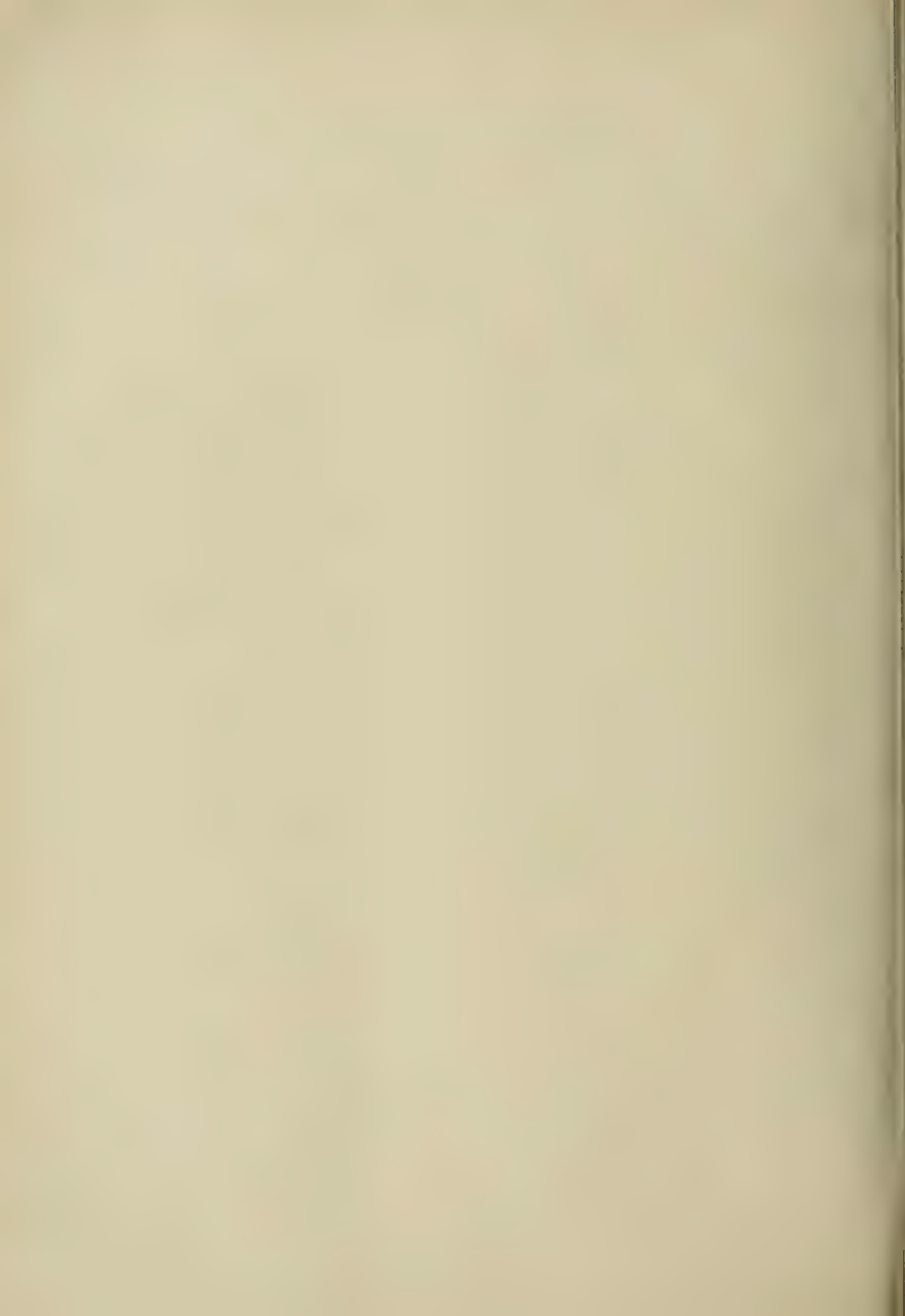


The nitrogen of monoamidocompounds was calculated from the difference.

### CONCLUSION.

From the results of the above two experiments, the following conclusion is drawn:

1. An addition of superphosphate to night soils is very recommendable method to decrease the loss of nitrogen as well as the volatilization of ammonia during the storage of excrements.
  2. These effects of superphosphate are, chiefly, due to the action of monocalcium phosphate contained in it.
  3. Gypsum and kainit are not so effective in regard to fixing ammonia.
  4. The diminution of the loss of nitrogen by the addition of superphosphate and monocalcium phosphate is caused partly to the fact that they diminish the putrefaction of albuminoids in night-soil.
  5. Since superphosphate diminishes the decomposition of albuminoids in night-soil, its addition can not be recommended in practice, especially not in colder climates, when the quick fermentation of night-soil is required for successful manuring<sup>6</sup>.
  6. It is true that the manurial action of night-soil is very much retained by the addition of superphosphate in the cold districts.
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## On the Manurial Value of Various Organic Phosphoric Compounds.

BY

K. Asō and T. Yoshida.

It is generally assumed that nitrogen, phosphoric acid and potash are the three most essential ingredients of manures and that the efficacy of manures chiefly depends upon the quantities of these three fertilising elements present in them and upon the degree of their availability. As for nitrogen e.g. ammoniacal, nitric and organic nitrogen, and as for phosphoric acid, water-soluble, citrate-soluble and insoluble phosphoric acid have been distinguished.

In recent times, however, various organic phosphoric compounds have been found in plants as well as in animals. Besides nuclein and lecithin also phytin was found widely distributed in the vegetable kingdom. Moreover, inosic acid, jecorin, kephalin and myelin were found in the animal body. These, as well as tricalcium phosphate, so important in higher animals are absent in plants, while nuclein (as nucleoprotein), lecithin and phytin play a great rôle in them.

Since plants are often used as manures, it is of importance to investigate the efficacy of the phosphoric acid in these different organic forms.

From the chemical point of view, nuclein would be more difficultly decomposed and therefore less available than lecithin and phytin. In water culture, Stoklasa<sup>1</sup> showed that lecithin is available to oats, but its manurial value is far below that of monocalcium phosphate, as shown by the following figures:

1. J. Stoklasa: Die Assimilation des Lecithins durch die Pflanze: Sitz.-Ber. Wiener Ak. 1907.

	No $P_2O_5$	$CaH_4(PO_4)_2$	Lecithin.
Total weight of dried plants... ..	2.09 g.	29.87 g.	20.52 g.
Weight of dried grains ... ..	—	7.45 g.	4.25 g.

Prof. U. Suzuki and Takaishi<sup>2</sup> in our laboratory made experiments with barley in sand and soil cultures to observe the availability of phytin<sup>3</sup> and the results were as follows:

#### SAND CULTURE.

	No $P_2O_5$	$Ca_2H_2(PO_4)_2$	Phytin.
Average length of plants.... ..	39 cm.	83 cm.	82 cm.
Total harvest (air-dried) ... ..	5 g.	23 g.	23 g.

#### SOIL CULTURE.

	No manure.	No $P_2O_5$	$Ca_2H_2(PO_4)_2$	Phytin.
Average length of plants ... ..	23 cm.	26 cm.	58 cm.	41 cm.
Total harvest (air-day) ... ..	2 g.	2 g.	7 g.	4 g.

These results show that the manurial value of phosphoric acid in the form of phytin is almost equal to that of diacalciumphosphate in sand culture, but the former is far behind the latter in soil culture.

Since, in Japan, various vegetable manures such as green manures, rice-brans, oil-cakes, straws etc are most widely used by practical farmers, the practical interest of the question induced me to the following experiments.

#### FIRST EXPERIMENT.

This experiment was carried out to compare the manurial value of various organic and inorganic phosphoric compounds. The soil serving for this experiment was a humus loam of a slightly acid reaction, which was exhausted by continuous cultivation without any manures for seven years.

Each pot containing 2.5 kilo, soil was manured as follows:

2. U. Suzuki, a. M. Takaishi: Journal of Agricultural Society, Japan. No. 323.
3. Phytin was prepared from rice-bran.

	Acidic manures. per pot.		Basic manures. per pot.
Ammonium sulphate... ..	1 g.	Sodium nitrate... ..	1.3 g.
Potassium sulphate ... ..	4.3 g.	Potassium carbonate ...	1 g.

Various phosphoric compounds were added as the following table shows, the quantity of  $P_2O_5$  applied per pot being 0.396 grams.

	Per pot.
Sodium phosphate ... ..	1 g.
Lecithin ... ..	5.107 g.
Phytin ... ..	0.845 g.
Nuclein ... ..	23.557 g.
Aluminium phosphate ... ..	0.681 g.
Ferrie phosphate ... ..	0.842 g.
Tricalcium phosphate ... ..	0.865 g.

The phytin was prepared from rice-brans as follows: the rice-bran was boiled with 95% alcohol twice after extracting with ether and the dried residue was repeatedly extracted with 0.2% hydrochloric acid. To the hydrochloric extract, 95% alcohol was added and the white precipitate obtained was washed with alcohol and ether, dissolved in 0.2% hydrochloric acid, reprecipitated with alcohol and washed as before. The white powder prepared in this way, contained 45.86%  $P_2O_5$ . The nuclein used, was prepared from beer-yeast in the usual way, and contained 1.68%  $P_2O_5$ .

The lecithin had been prepared by König & Co. and contained 7.75%  $P_2O_5$  showing that this was tolerably pure.

Sodium phosphate, ferrie phosphate, aluminium phosphate and tricalcium phosphate were chemically pure.

On Nov. 7, these compounds were mixed with the soil respectively, and barley was sown, seven seeds in each pot. The young plants were reduced afterwards to three of equal size in each pot. After germination, a great difference of development was observed and on March 14, the following measurement was made:

## AVERAGE LENGTH OF PLANTS.

	Acidic manure, cm.	Basic manure, cm.
Lecithin ... ..	27.3	20.4
Phytin ... ..	9.3	11.1
Nuclein ... ..	No test.	9.3
Sodium phosphate ... ..	16.2	15.9
Ferric phosphate ... ..	12.0	0.6
Aluminium phosphate ... ..	12.3	10.2
Tricalcium phosphate ... ..	23.0	21.0
No $P_2O_5$ ... ..	8.1	7.8
No manure ... ..	7.8	0

On May 13, a photograph was taken; the plants were harvested June 16, with the following result:

	Manures.	Total weight, air-dry, g.	Ears, air-dry, g.	Grains, air-dry, g.
Lecithin ... ..	{ Acidic	12.9	6.1	4.8
	{ Basic	11.7	5.7	4.5
Phytin ... ..	{ Acidic	3.4	1.2	1.0
	{ Basic	2.5	0.7	0.5
Nuclein ... ..	{ Acidic	No test.		0.45
	{ Basic			
Sodium phosphate ... ..	{ Acidic	8.7	4.6	3.5
	{ Basic	6.0	2.5	1.8
Ferric phosphate ... ..	{ Acidic	4.0	2.0	1.6
	{ Basic	3.5	1.3	0.8
Aluminium phosphate ... ..	{ Acidic	3.1	0.6	0.3
	{ Basic	3.0	1.0	0.5
Tricalcium phosphate ... ..	{ Acidic	10.4	4.7	3.0
	{ Basic	9.9	—	3.2
No $P_2O_5$ ... ..	{ Acidic	0.7	0	0
	{ Basic	1.4	0	0
No manure ... ..		0.5	0	0

From these results it is clear that lecithin is easily decomposed in the soil and furnishes phosphoric acid available for plants. The remarkable result with lecithin might be supposed to be partly caused by the nitrogen contained in lecithin, but this is improbable, as there was surely sufficient nitrogen in the general manure. Tricalcium phosphate<sup>4</sup> exerted

4. This was precipitated tricalcium phosphate.

in this experiment a very favorable influence. The manurial value of phytin was far less than that of sodium phosphate and almost equal to that of ferric and aluminium phosphate. Perhaps, phytin will be changed to insoluble compounds in soils, since the iron and aluminium compounds of phytin are insoluble. As for nuclein, the author made the experiment only with basic manures, since nuclein is soluble in alkalies and not in acids; nevertheless its manurial value was very small, showing that the decomposition by bacteria in the soil is rather slow.

Among organic phosphoric compounds in plants, the manurial value of lecithin is excellent while those of phytin and nuclein are far less. But, the content of lecithin in plants is generally very small, while that of phytin much larger. This latter plays also an important part in certain vegetable manures as the following table shows<sup>5</sup>:

## IN 100 PARTS OF DRY MATTER.

	Total $P_2O_5$	$P_2O_5$ in lecithin.	$P_2O_5$ in phytin.	$P_2O_5$ in nuclein.
Soybean cake... ..	1.311	0.114	0.640	0.236
Rape cake ... ..	2.251	0.091	0.873	0.204
Red clover ... ..	0.554	0.150	0.300	0.050

The inferior value of phosphoric acid in vegetable manures to that in animal manures<sup>6</sup> is decidedly explained by these results.

However, since there exists an enzyme<sup>7</sup> called phytase which splits phytin with the production of inorganic phosphoric compounds, and which is present in various plants, the phytin applied in the form of press cakes will yield a somewhat better result than when applied in the pure state, especially, when the cakes were left to putrefy before application.

## SECOND EXPERIMENT.

This experiment was carried out to decide which part of the phosphoric acid in rice-bran has the highest manurial value. Each pot containing

5. Cf. the article of Tsuda, this Journal.

6. Cf. Nagaoka's result with paddy rice, these bulletins, Vol. IV.

7. U. Suzuki and K. Yoshimura: these bulletins, Vol. VII, No. 4.

2.5 kilo soil, was manured Nov. 8, with 3 g. ammonium sulphate and 2 g. potassium carbonate. Besides, 10 g. of rice-bran treated in various ways were added to the different pots. Seven seeds of barley were sown in each, and the young plants reduced afterwards to three of equal size. On March 14, the following measurement was made:

	Average length of plants. cm.
Original rice-bran ... ..	8.9
Extracted with ether ... ..	12.1
" " " and alcohol... ..	15.1
" " " alcohol and 0.2% HCl... ..	8.4
" " " " " 10% HCl... ..	8.4
Sodium phosphate (equivalent to original rice-bran) .. ..	13.2
No $P_2O_5$ ... ..	7.6
No Manure ... ..	7.5

On June 16, the plants were harvested and weighed in air-dry state with the following result:

	Total weight. g.	Ears. g.	Grains. g.
Original rice-bran ... ..	3.3	1.1	0.65
Extracted with ether ... ..	4.7	1.5	1.0
" " " and alcohol ... ..	4.8	1.9	1.4
" " " " " and 0.2% HCl. ... ..	0.9	—	—
" " " " " and 10% HCl. ... ..	0.8	—	—
Sodium phosphate... ..	2.3	0.6	0.35
No $P_2O_5$ ... ..	0.9	—	—
No manure ... ..	0.8	—	—

A similar experiment was made with oats using rape-cake as manure, with the following result:

	Total weight. g.	Straw. g.	Grains. g.
Original rape-cake. ... ..	$\left\{ \begin{array}{l} 3.3 \\ 2.5 \end{array} \right.$	$\left\{ \begin{array}{l} 1.8 \\ 1.5 \end{array} \right.$	$\left\{ \begin{array}{l} 1.5 \\ 1.0 \end{array} \right.$
Extracted with ether ... ..	$\left\{ \begin{array}{l} 5.8 \\ 4.5 \end{array} \right.$	$\left\{ \begin{array}{l} 2.5 \\ 2.2 \end{array} \right.$	$\left\{ \begin{array}{l} 3.3 \\ 2.3 \end{array} \right.$
Extracted with ether and alcohol ... ..	$\left\{ \begin{array}{l} 5.5 \\ 5.0 \end{array} \right.$	$\left\{ \begin{array}{l} 2.5 \\ 2.4 \end{array} \right.$	$\left\{ \begin{array}{l} 3.0 \\ 2.6 \end{array} \right.$
Extracted with ether, alcohol and 0.2% HCl. ... ..	$\left\{ \begin{array}{l} 0.35 \\ 0.38 \end{array} \right.$	$\left\{ \begin{array}{l} 0.20 \\ 0.30 \end{array} \right.$	$\left\{ \begin{array}{l} 1.5 \\ 0.8 \end{array} \right.$



Extracted with ether, alcohol and 10% HCl...	$\begin{cases} 0.3 \\ 0.4 \end{cases}$	$\begin{cases} 0.2 \\ 0.3 \end{cases}$	$\begin{cases} 0.1 \\ 0.1 \end{cases}$
Sodium phosphate ... ..	$\begin{cases} 10.6 \\ 8.7 \end{cases}$	$\begin{cases} 5.1 \\ 4.2 \end{cases}$	$\begin{cases} 5.5 \\ 4.5 \end{cases}$
No $P_2O_5$ ... ..	$\begin{cases} 0.00 \\ 0.80 \end{cases}$	$\begin{cases} 0.35 \\ 0.30 \end{cases}$	$\begin{cases} 0.55 \\ 0.50 \end{cases}$
No manure ... ..	$\begin{cases} 0.42 \\ 0.40 \end{cases}$	$\begin{cases} 0.35 \\ 0.30 \end{cases}$	$\begin{cases} 0.70 \\ 0.10 \end{cases}$

These results show that rice-bran and rape cake exert a better manurial effect after the fat had been extracted by ether and alcohol. Evidently the fatty matter surrounds the particles of cake and prevents the root hairs to exert their absorptive power.

Since phytin is soluble in 0.2% hydrochloric acid, the residue after extracting with ether, alcohol and 0.2% hydrochloric acid has lost almost all value as phosphatic manure<sup>8</sup>. Hence it must be concluded that, to phytin is due the chief manurial value of phosphoric acid in press cakes.

### THIRD EXPERIMENT.

In this experiment, various residues obtained from rice-bran as before were analysed separately and the quantities corresponding to one gram of sodium phosphate were used in each pot. The contents of  $P_2O_5$  and the quantities of these residues were as follows:

	$P_2O_5$ %	Quantity used, per pot. g.
Original rice-bran... ..	6.758	5.87
Extracted with ether ... ..	8.684	4.56
Extracted with ether and alcohol ... ..	8.824	4.49
Extracted with ether, alcohol and 0.2% HCl.	4.157	9.53
Extracted with ether, alcohol and 10% HCl...	2.525	15.68

Each pot containing 2.5 kilo soil was manured on Nov. 18, and seeds of pea, rape, barley were sown, seven in each pot. The young plants were reduced to two of equal size in the case of pea, to four in the case of rape and barley.

8. Of course, all hydrochloric acid was removed by well-washing, until no acid was perceived in the filtrate.

On April 14, a photograph was taken, and the plants were harvested May 30, with the following result:

## PEA.

	Total weight.	Stems.	Fruits.
	g.	g.	g.
Original rice-bran .. .. .	1.85	0.68	1.18
Extracted with ether ... ..	4.67	1.27	3.40
Extracted with ether and alcohol ... ..	3.61	1.59	2.02
Extracted with ether, alcohol and 0.2% HCl. ...	0.91	0.52	0.39
Extracted with ether, alcohol and 10% HCl. ...	1.04	0.56	0.48
Sodium phosphate ... ..	7.64	1.55	6.09
No $P_2O_5$ ... ..	0.47	0.47	—

## RAPE.

	Total weight.	Stems.	Fruits.
	g.	g.	g.
Original rice-bran... ..	2.33	1.27	1.06
Extracted with ether ... ..	3.10	1.66	1.44
Extracted with ether and alcohol ... ..	3.50	1.95	1.55
Extracted with ether, alcohol and 0.2% HCl. ...		No development.	
Extracted with ether, alcohol and 10% HCl. ...		"	
Sodium phosphate ... ..	6.00	3.70	2.30
No $P_2O_5$ ... ..		No development.	

The barley was harvested on June 16 and weighed in air-dry state as follows:

	Total weight.	Ears.	Grains.
	g.	g.	g.
Original rice-bran... ..	1.4	—	—
Extracted with ether ... ..	1.6	—	—
Extracted with ether and alcohol ... ..	3.6	1.3	0.9
Extracted with ether, alcohol and 0.2% HCl. ...	1.1	—	—
Extracted with ether, alcohol and 10% HCl. ...	0.8	—	—
Sodium phosphate ... ..	7.7	2.4	1.4
No $P_2O_5$ ... ..	0.9	—	—
No PEARL ... ..	0.9	—	—

Although the quantity of  $P_2O_5$  was the same in all pots, yet the residue obtained by extracting with ether and alcohol yielded best harvest which coincides with the results obtained in the second experiment. The

residue containing only nuclein as phosphoric compound, obtained by extracting rice-bran with ether, alcohol and 10% hydrochloric acid has no immediate manurial value as a phosphatic manure, even in large doses, which agrees with results of the first and the second experiments.

### CONCLUSION.

1. Among the organic phosphoric compounds used in these experiments, the manurial value of lecithin was highest, phytin come next and nuclein last.

2. The manurial value of lecithin was not lower than that of sodium phosphate, that of phytin was nearly equivalent to that of ferric or aluminium phosphate and that of nuclein was very low.

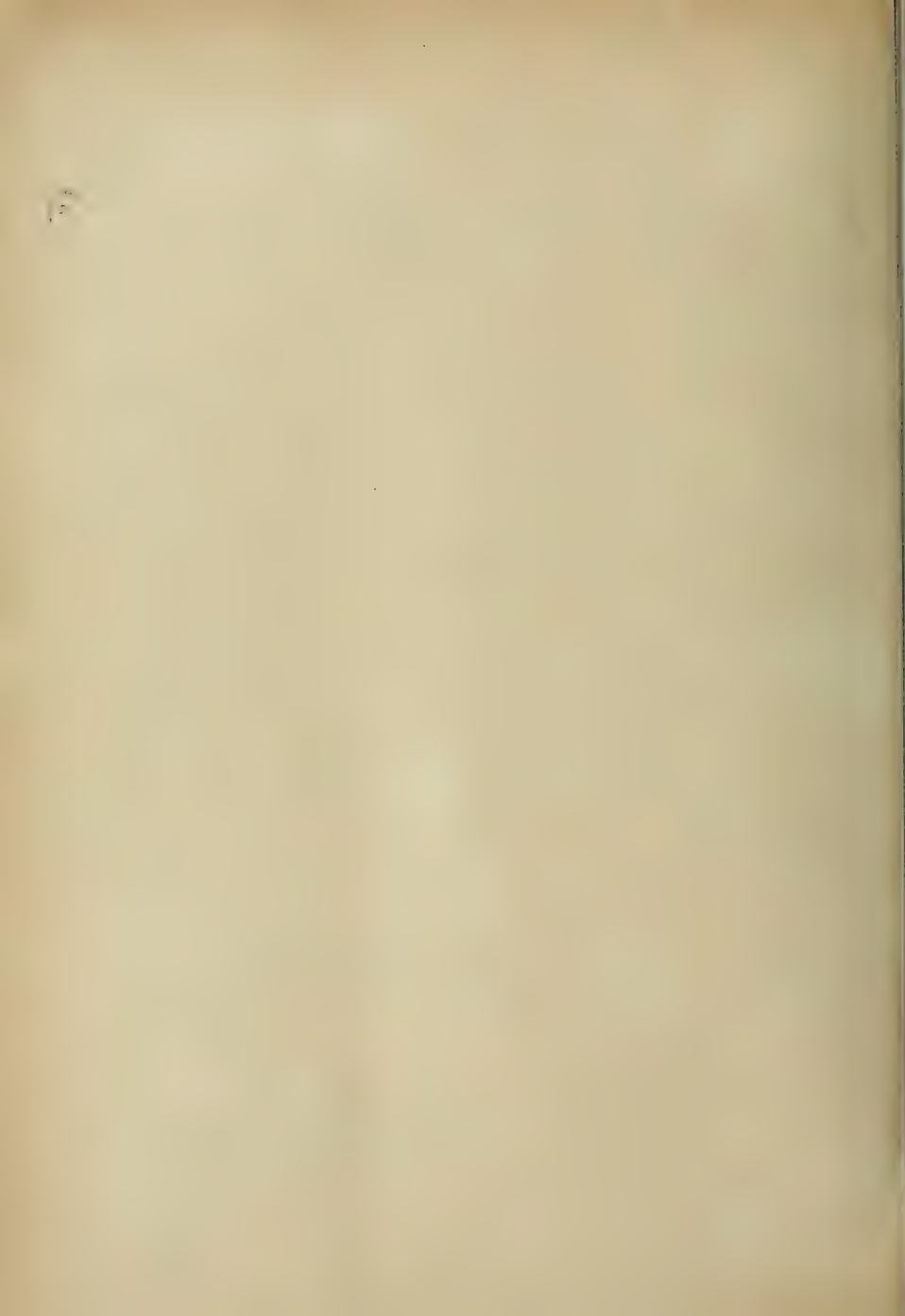
3. The most essential phosphoric compound in vegetable manures is phytin.

4. As phytin is easily transformed in soils into insoluble ferric and aluminium phosphate it is recommendable to use vegetable manures in a putrefied state to render the phosphoric acid available.

5. In the analysis of manures, it is absolutely necessary to pay attention to the different organic phosphoric compounds.

6. Further experiments with various organic manures along this line and with different soils are still desirable.

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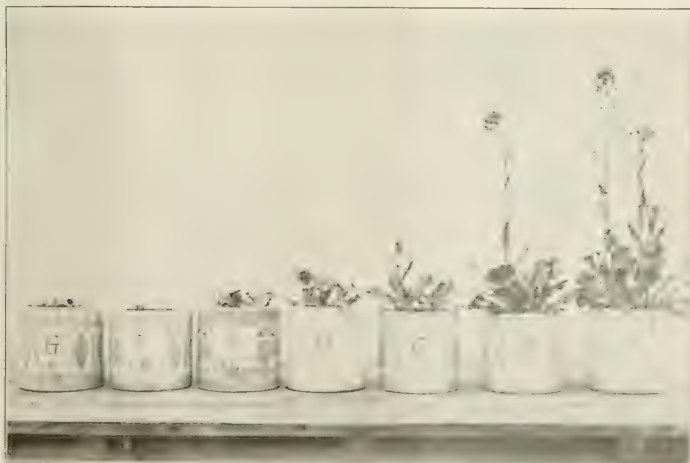




$\text{Na}_3\text{HPO}_4$   $\text{Ca}_3(\text{PO}_4)_2$   $\text{Fe}_2(\text{PO}_4)_2$   $\text{Al}_2(\text{PO}_4)_3$  Lecithin Phytin Nuclein No  $\text{P}_2\text{O}_5$ , No manure

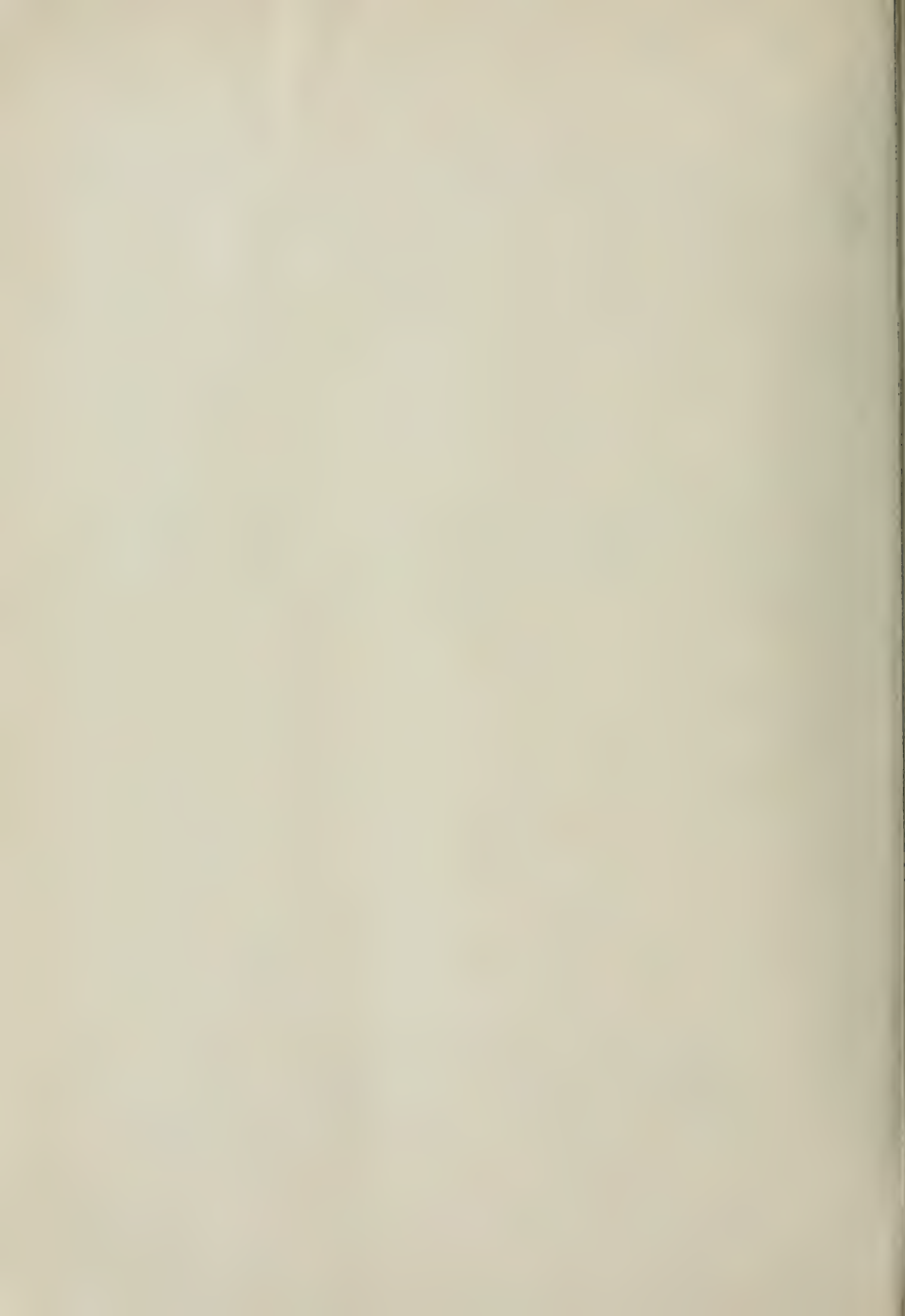
This plate shows the result of the first experiment.

## PL. II.



No  $\text{P}_2\text{O}_5$     Extracted with ether, alcohol and 10% HCl.    Extracted with ether, alcohol and 0.2% HCl.    Original rice-bran.    Extracted with ether.    Extracted with ether and alcohol.     $\text{Na}_2\text{HPO}_4$

This plate shows the result of the third experiment.



# On the Availability of Phosphoric Acid in Various Forms in Herring-guano.

BY

R. Mitsuta.

There exist various forms of phosphoric acid in herring-guano,<sup>1</sup> such as calcium phosphate, lecithin, phytin, nuclein and other inorganic phosphates. But as to the availability of these different phosphoric compounds, some investigation seemed desirable. Prof. Aso<sup>1</sup> compared the manurial value of various organic phosphoric compounds derived from rice-bran, rape-cake and yeast.

For my experiment served eight pots, each containing 2.5 Kilo soil,<sup>2</sup> manured with 2g. potassium carbonate and 3g. sodium nitrate. These large doses were necessary to eliminate the effect of potash and nitrogen of the herring-guano. Besides, five grams of herring-guano treated in various ways were added in each pot:

Pot.	Extracted with	Removed
A.	Original (not extracted).	—
B.	Ether.	Oils and a part of lecithin.
C.	Ether and alcohol.	Oils and lecithin.
D.	Ether, alcohol and hot water.	<div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 2em; vertical-align: middle; margin-right: 5px;">{</div> Oils, lecithin and phosphates soluble in water. </div>
E.	Ether, alcohol and 0.2% HCl.	
F.	Ether, alcohol and 10% HCl.	
G.	No phosphatic manure added.	—
H.	4.3g. sodium phosphate corresponding to 5g. herring-guano which contained 4.176% P <sub>2</sub> O <sub>5</sub> .	—

1. Cf. This Bulletin.

2. This soil was a humus loam of a field unmanured for eight years.

On Nov. 26, seven seeds of barley were sown in each pot, and the young plants reduced to three afterwards. After the germination, a great difference in development was observed. On May 13, a photograph was taken and the following measurement made:

Pot	1st plant		2nd plant		3rd plant	
	Average length cm.	Number of branches	Average length cm.	Number of branches	Average length cm.	Number of branches
A ... ..	7.2	3	7.0	3	6.6	2
B ... ..	6.0	3	5.3	2	4.5	3
C ... ..	5.1	3	5.1	3	5.1	3
D ... ..	4.5	1	4.2	2	3.9	2
E ... ..	1.5	1	1.5	1	1.2	1
F ... ..	0.3	1	0.4	1	0.3	1
G ... ..	—	1	—	1	—	1
H ... ..	5.4	4	5.1	3	5.4	3

On June 26, the plants were harvested and weighed in air-dry state:

Pot.	Total weight g.	Number of Ears.	Weight of Ears. g.
A ... ..	8.5	4	3
B ... ..	7	5	2.5
C ... ..	7	5	1.5
D ... ..	5	4	1
E ... ..	1.2	—	—
F ... ..	—	—	—
G ... ..	—	—	—
H ... ..	7.7	4	2.4

In this case, treatment with ether or ether and alcohol had not increased the manurial value, but decreased. Here the cake of commerce had been deprived of the most of its oil content<sup>3</sup> and it was chiefly lecithin, perhaps from lecithalbumen, which had been extracted.

The great difference between D and E was caused by that part of phosphoric acid which is soluble in dilute acids. Since the content of

3. For this experiment, Shimckasu which is a pressed residue deprived of the most part of its oil-content, was used.



phytin in herring-guano is very small, the phosphoric acid soluble in dilute acid might be present in inorganic forms, chiefly as calcium phosphate.<sup>4</sup> Some development of plants in E shows that there remained still a part of tricalciumphosphate in bones unextracted, the case being quite different from that of vegetable manures.<sup>5</sup> Nuclein in herring-guano has no immediate manurial value as generally assumed.

Lastly, we conclude that the principal part of phosphoric acid serving as phosphatic manure in fish-guanos is of inorganic nature, chiefly consisting of calcium phosphate. Lecithin and phosphates soluble in water exert here also a certain rôle. Hence a great difference regarding the manurial value of phosphoric acid in animal and vegetable manures was thus established.

4. Cf. The articles of Funatsu and Tsuda. (These Bulletins).

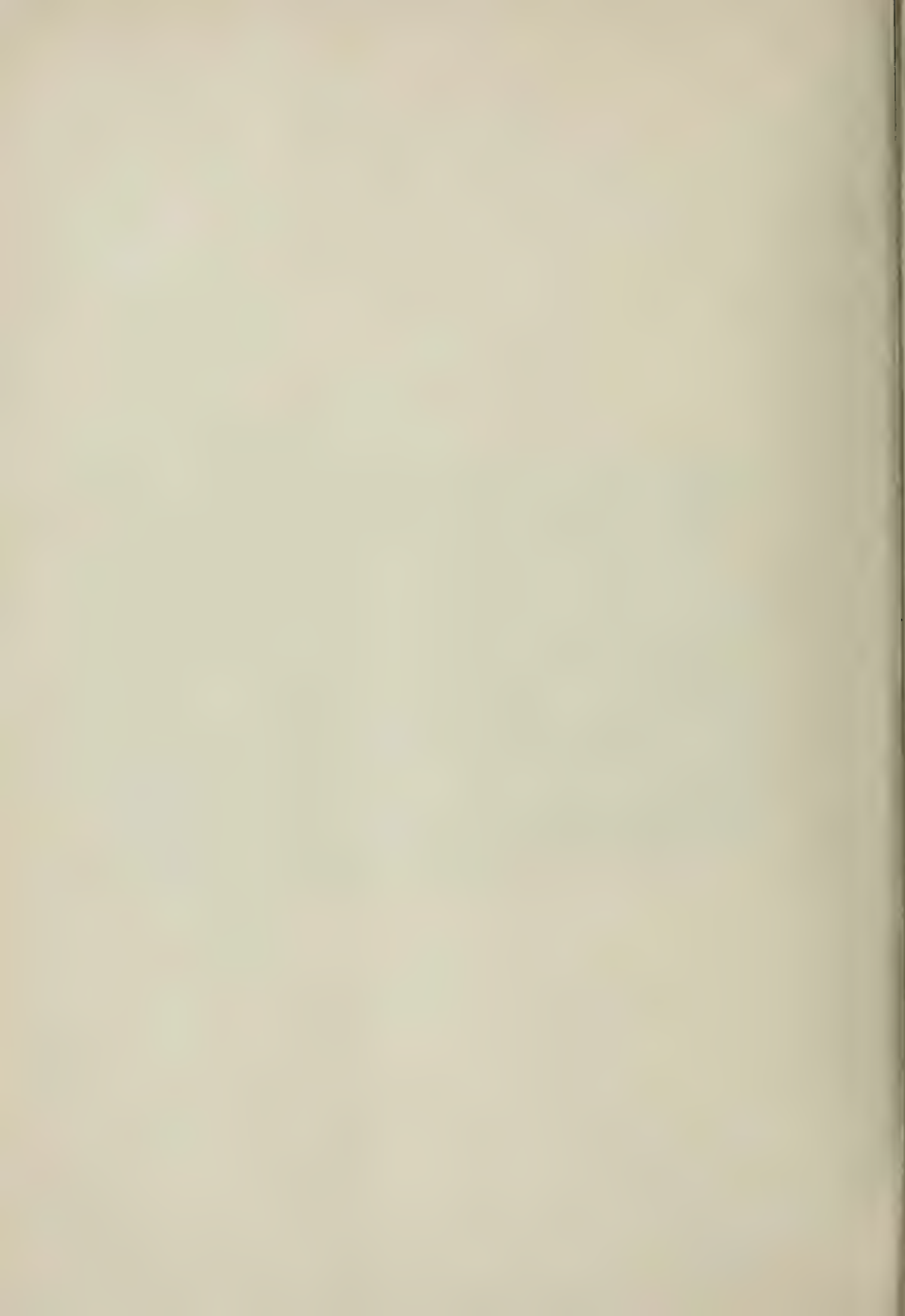
5. Asō. This Journal.

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Plato II.





## On the Different Forms of Phosphoric Acid in Organic Manures.

BY

S. Tsuda.

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It is a well-known fact that phosphoric acid is one of the three essential manurial elements, but there has been no systematic research to determine the different forms under which it occurs in different organic substances. Fumatsu<sup>1</sup> determined phosphoric acid in the form of lecithin and nuclein, and in a form soluble in dilute hydrochloric acid in several manure cakes. Prof. U. Suzuki and Yoshimura<sup>2</sup> have found that phytin is widely distributed in vegetable matters, forming the chief part of the phosphoric compounds of the plant body, and the object of the present paper is the quantitative determination of different forms of phosphoric acid in several organic manures of vegetable as well as animal origin.

To determine phosphoric acid in the form of lecithin, 50g. air-dry sample was extracted for twenty hours with ether by means of Soxhlet's apparatus. The residue was boiled for six hours with absolute alcohol, using a reverted cooler, and the extraction was repeated after pouring off the alcoholic liquid. After evaporating these ether and alcoholic extracts to dryness they were fused with potassium nitrate and sodium carbonate, and the fused mass was dissolved in nitric acid. The phosphoric acid was then determined by molybdic method.

The residue obtained from the alcoholic extraction was dried and

1. Bul. College of Agric. Tokyo, Vol. VII. No. 3.

2. Bul. College of Agric. Tokyo, Vol. VII. No. 4.

put into a flask, and extracted with 500 c.c. of 0.2% hydrochloric acid at the room temperature for six hours the flask being shaken from time to time. After filtering, the extraction was repeated with the residue and the filtrate was mixed with the first. To a part of the hydrochloric filtrate ammonia was first added to make the reaction slightly alkaline, then nitric acid in excess, and the phosphoric acid of this extract in the form of inorganic salts was determined by molybdic method. To determine the phosphoric acid in the form of phytin, another part of the hydrochloric extract was evaporated in a large platinum basin and the residue was well fused with potassium nitrate and sodium carbonate. After extracting the fused mass with nitric acid, molybdic method was applied. The difference between the total and the inorganic phosphoric acid of the original hydrochloric acid is the phosphoric acid in the form of phytin.

The residue of the 0.2% hydrochloric acid extraction was dried and extracted with 500 c.c. of 5% hydrochloric acid in the same manner as before and its phosphoric acid, in the form of both organic and inorganic compounds, was determined. The last residue was dried and fused with the fusing-mixture, dissolved in dilute nitric acid, and molybdic method was applied to determine the phosphoric in the form of nuclein. The results obtained are shown in the following table.

## IN 100 PARTS OF DRY MATTER.

	Soybean cake.	Rape seeds cake.	Red clover hay (before flowering)
Total $P_2O_5$ ... ..	1.311	2.251	0.554
$P_2O_5$ sol. in ether and alcohol (as Lecithin) ... ..	0.114	0.091	0.050
$P_2O_5$ sol. in { inorganic ... ..	0.050	0.050	Trace
0.2% Hcl. { organic (as phytin) ... ..	0.640	0.873	0.300
$P_2O_5$ sol. in { inorganic ... ..	0.040	0.099	0.070
5% Hcl. { organic ... ..	0.120	0.931	0.084
$P_2O_5$ in the last residue (as nuclein)...	0.236	0.204	0.050

	Herring guano.	Steamed bone dust	Pressed cake of pupa of Silk-worms	Crab shells.
Total $P_2O_5$ ... ..	4.670	25.060	1.350	3.23
$P_2O_5$ sol. in ether and alcohol (as Lecithin). ... ..	0.310	0.023	0.043	0.023
$P_2O_5$ sol. in inorganic... ..	1.894	5.534	1.039	0.300
0.2% HCl organic ... ..	0.860	trace	trace	0.151
$P_2O_5$ sol. in inorganic... ..	0.372	18.859	0.090	2.264
5% HCl. organic ... ..	0.648	0.530	trace	0.200
$P_2O_5$ in the last residue (as nuclein) ... ..	0.583	0.112	0.169	0.302

These results show distinctly the difference between animal and vegetable manures in the relative amount of the different forms of phosphoric acid, the former containing it mostly in the form of inorganic compounds and the latter mostly as organic compounds.

In vegetable manures, phosphoric acid is present principally in the form of phytin<sup>3</sup> and the amount of nuclein is comparatively small. Lecithin is also contained in small quantities, the phosphoric acid in this form being always less than 10% of the whole. Again, as inorganic compounds phosphoric acid is present only in traces in certain cases.

On the other hand, since tricalcium phosphate is the principal ingredient of bones, most of the phosphoric acid of animal manures containing bones is the part soluble in 5% hydrochloric acid; but in the case of herring guano, the phosphoric acid soluble in 0.2% hydrochloric acid is more than the former, owing to the presence of a large amount of flesh.<sup>4</sup>

Phosphoric acid is contained in crab shells in nearly the same forms as in bones, while in the pupa of *bombyx mori* silk-worms it is present in an inorganic form easily soluble in dilute hydrochloric acid (.2%)<sup>5</sup>.

3. Phosphoric acid as phytin which has not been completely extracted with 0.2% hydrochloric acid owing to the presence of protein and other ingredients may be contained in the organic phosphoric acid extracted with 5% hydrochloric acid.

4. Fresh and meat contain potassium phosphates and certain organic phosphoric compounds besides nuclein and lecithin.

5. This is a very interesting fact and further study will be made in this line.

The great difference in phosphoric compounds between animal and vegetable manures proved above probably explains to some extent the different value of animal and vegetable manures, the former being always superior, as Prof. Nagaoka<sup>6</sup> has shown.

6. *Ibid.* College of Agric. Tokyo, Vol. VI, No. 3.

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## On the Influence of Different Ratios of Lime to Magnesia on the Growth of Rice II.

BY

K. Asō.

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In my first communication on this subject<sup>1</sup> it was shown experimentally that the ratio  $\text{CaO}:\text{MgO}=1$  in the soil is the most favorable for rice under the condition that both these bases are about equally available. In that experiment the manure applied was of alkaline nature; phosphoric acid having been applied as  $\text{Na}_2\text{HPO}_4$  and potash as carbonate. Under this condition the injurious influence of an excess of magnesia is generally not so intense as in the presence of a manure of acid character. To observe the growth of rice on application of an acid manure with varying ratios of lime to magnesia, I selected this time double superphosphate as the source of  $\text{P}_2\text{O}_5$ ; potash was further applied as sulphate, and nitrogen half as ammonium sulphate and half as sodium nitrate. Lime and magnesia were added in one series<sup>2</sup> as sulphates and in a second series as carbonates,—in varying proportions.

Seven Wagner's porcelain pots were filled with 8 kilo of air-dry sifted soil taken from a paddy field which had not been cultivated for several years. The quantity of available lime and magnesia in this soil was determined by extracting the soil with cold 10% hydrochloric acid for 48 hours with the following result:

In 100 parts of dry soil;	
CaO	0.70
MgO	0.60

1. These Bulletins, Vol. VI, No. 2.

2. In my first experiment carried out three years ago, lime and magnesia were applied as the *natural* carbonates in a state of very fine powder.

The amounts of gypsum and magnesium sulphate, applied in series I, further those of the precipitated carbonates of lime and magnesia<sup>1</sup>, in further those of the precipitated carbonates of lime and magnesia<sup>3</sup>, in

## SERIES I.

Pots.	Quantity of $\text{CaSO}_4 + 2\text{H}_2\text{O}$ added, g.	Quantity of $\text{MgSO}_4 + 7\text{H}_2\text{O}$ added, g.	CaO : Mg O.
a	285.93	—	5 : 1
b	211.32	—	4 : 1
c	136.67	—	3 : 1
d	62.04	—	2 : 1
e	—	—	1 : 1 (nearly)
f	—	100.13	1 : 1.5
g	—	200.27	1 : 2

## SERIES II.

Pots.	Quantity of $\text{CaCO}_3$ added, g.	Quantity of basil $\text{MgCO}_3$ added, g.	Ca O : Mg O.
A	166.24	—	5 : 1
B	122.86	—	4 : 1
C	79.46	—	3 : 1
D	36.07	—	2 : 1
E	—	—	1 : 1 (nearly)
F	—	68.3	1 : 2
G	—	127.4	1 : 3

On July 15, the following compounds were applied to each pot as general manure:

Double superphosphate	5 g.
Potassium sulphate	10 g.
Ammonium sulphate	5 g.
Ammonium nitrate	5 g.

On July 16, three bundles of young rice plants, each bundle of 3 plants of equal size were transplanted from the seed bed into each pot. The plants were treated as is usual for paddy plants, and care was taken

3. The ratio  $\frac{0.7 \text{ CaO}}{0.6 \text{ MgO}}$  corresponds to  $\frac{1.16}{1}$ .

that no unnatural conditions should occur. The accompanying photograph was taken on September 7. On November 5, the plants were cut, left to become air-dry for several weeks and weighed.

Pots.	Grains, g.	Straw, g.	Total, g.
a	16.5	61.5	78.0
b	26.5	59.0	86.5
c	40.8	58.2	99.0
d	35.5	81.5	117.0
e	60.8	80.7	<b>141.5</b>
f	22.0	65.0	87.0
g	13.2	41.3	54.5
A	49.5	71.0	120.5
B	42.0	70.5	112.5
C	47.5	76.0	123.5
D	49.0	65.0	114.0
E	62.0	80.8	<b>142.8</b>
F	49.8	69.2	119.0
G	51.5	73.5	125.0

This result agrees well with my former experiment carried on with the application of lime and magnesia as natural carbonates. It shows that any change of the ratio  $\text{CaO} : \text{MgO} = 1$  leads to a decrease of the harvest. The unfavorable effects of the sulphates upon the yield were more marked than those of the carbonates, probably on account of the slightly acid reaction in the soil<sup>4</sup>.

4. It is true that the precipitated magnesium carbonate is much more available than the precipitated calcium carbonate, and the condition of equal availability for F and G is not fulfilled. But this objection does not apply to my former experiment.—Further the sulphates of lime and magnesia underwent of course more or less change in the soil.



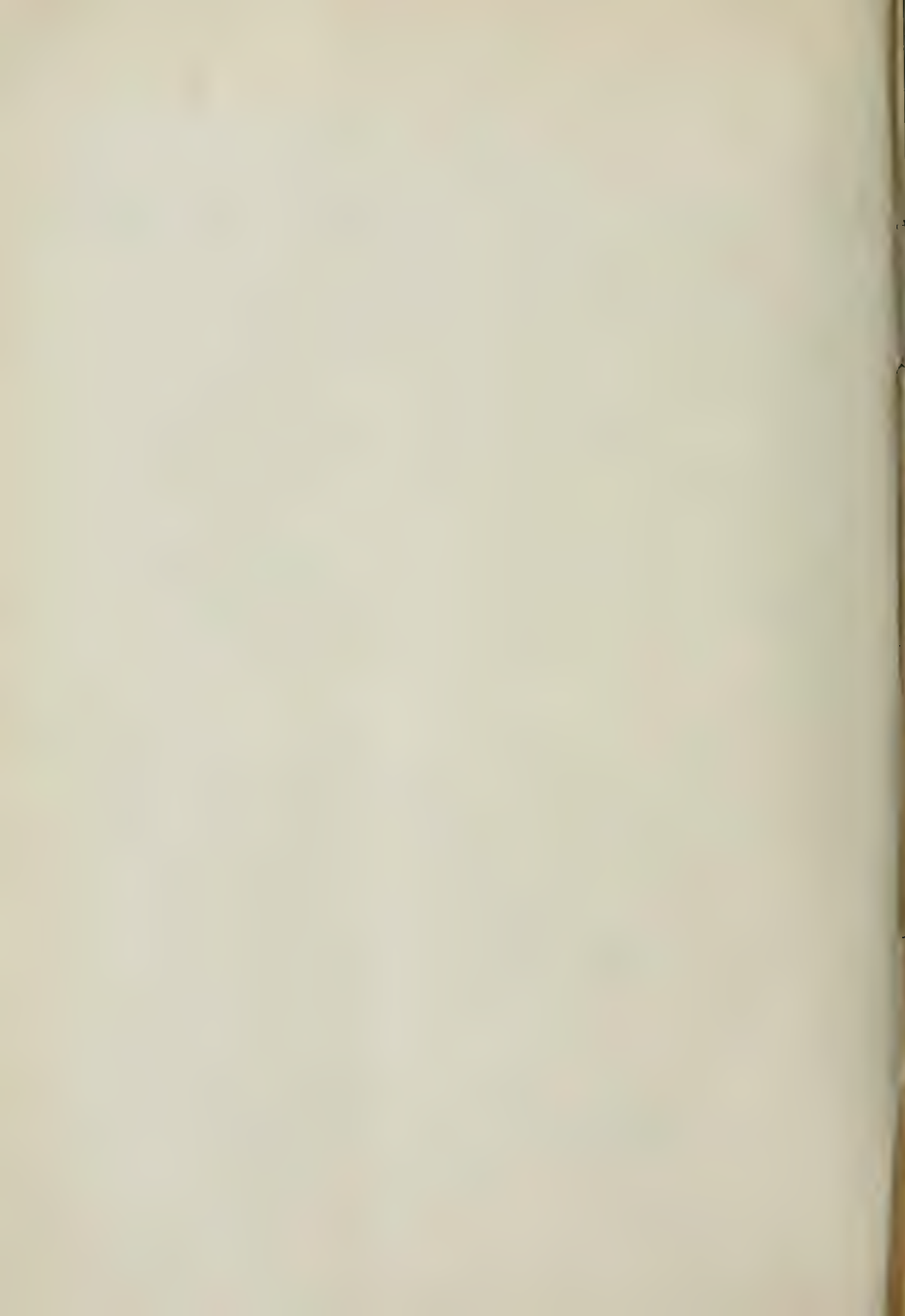


This plate shows the influence of different ratios of lime to magnesia with sulphates.



This plate shows the influence of different ratios of lime to magnesia with carbonates.

Left figures: instead of I, II, III, IV read  $A_1, A_2, A_3, A_4$ .  
Right figures: instead of a, b, c, d read  $B_1, B_2, B_3, B_4$ .



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## On the Influence of the Ratio of Lime to Magnesia upon the Yield in Sand Culture.

BY

K. ASO.

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Numerous experiments carried out here at this college as well as at the Central Experimental Station in Nishigahara have proved that the maximum yield depends, other things being equal, also upon a certain ratio of lime to magnesia absorbed by the plants. Some crops are more sensitive in this regard than others. That ratio—the lime-factor—varies with different crops, the variations ranging mostly between 1:1 and 4:1.

But there are some agriculturists who still cling to the opinion that the efficient factor in this case is not the unfavorable ratio, but the *absolute* excess of one base or the other. Any one who studies the question carefully on the basis of experiments must become convinced of the erroneousness of this opinion.

Above all, in regard to water cultures, the tests were carried out under the condition, that the sum of lime and magnesia was kept constant, while their relative amounts varied in different cases. It would be absurd to assume that 0.2% lime in water culture would be in itself injurious. Barley plants remained quite healthy for several weeks in culture solutions with 0.5% calcium nitrate in the absence of magnesia and died finally after 61 days from mere inanition<sup>1</sup>.

In the control case with magnesium nitrate and no lime the plants died in 10 days. The inanition in the former case as well as the poisonous action in the latter case was avoided by a proper ratio of lime to magnesia.

1. The discovery of Willstätter, that magnesium is essential for the formation of chlorophyll would furnish here the simplest explanation.

Further the experiments of Maki and Tanaka<sup>2</sup> have proved that after overliming the original fertility of the soil can be restored by the addition of magnesia. This would be impossible if only the absolute amount of lime and not the ratio of lime to magnesia were the cause of the depression. On the other hand it has also been shown that the injurious effect due to an excess of magnesia in the soil can be avoided by a proper dose of lime. Hence it is of fundamental importance to bring about a favorable ratio of lime to magnesia in the soil,—or, in case of a very different degree of availability of the lime and magnesia compounds to provide that the two bases be absorbed by the plants in suitable ratio<sup>3</sup>. Recently two Italian authors, L. Bernardini and G. Corso, of the Agricultural Experiment Station of Portici, have contributed further experimental data in regard to this problem<sup>4</sup>.

Rye and maize were grown in culture solution, and rye, maize and pea in pots and maize also in field culture with varying ratios of lime to magnesia, with the result, that the ratio 1:1 proved the best for rye, 2:1 for maize and 3:1 for pea. The authors conclude with the words:

“Queste prime ricerche confermano i risultati ottenuti dagli sperimentatori giapponesi e dimostrano quanta influenza abbiano sullo sviluppo delle piante i diversi rapporti fra calce e magnesia nel terreno.”

The writer has carried out some further experiments on this question in sand culture, in which the two bases, lime and magnesia, were offered to the plants in different quantities, but always in the same ratio. Oats and adzuki-beans (*Phaseolus mungo*) were selected for these tests. Our previous experiments have indicated that for leguminous plants the most favorable ratio is about 3:1, and that for oats it lies between 1:1 and 2:1. The ratios applied now were:

2. Bul. College of Agric. Tokyo, Vol. VII, No. 1.

3. Cf. Daikuhara's experiments with lime as carbonate and magnesia as sulphate. (Bul. Imp. Centr. Agric. Exp. Station Japan Vol. I. No. 1.)

4. Intorno all'influenza di vari rapporti fra calce e magnesia sullo sviluppo delle piante, Portici, 1907.



$$\frac{\text{Ca O}}{\text{Mg O}} = \frac{0.5}{1} : \frac{1}{1} ; \frac{2}{1} ; \frac{4}{1}$$

Two series of pots, each containing 2½ kilo sand, were prepared, in one of which the absolute amounts of the two bases were five times those of the other, namely:

	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>
$\frac{\text{Ca O}}{\text{Mg O}}$	$\frac{1\text{g.}}{2\text{g.}}$	$\frac{2\text{g.}}{2\text{g.}}$	$\frac{4\text{g.}}{2\text{g.}}$	$\frac{8\text{g.}}{2\text{g.}}$
	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>
$\frac{\text{Ca O}}{\text{Mg O}}$	$\frac{5\text{g.}}{10\text{g.}}$	$\frac{10\text{g.}}{10\text{g.}}$	$\frac{20\text{g.}}{10\text{g.}}$	$\frac{40\text{g.}}{10\text{g.}}$

Both bases were applied in the form of the natural carbonates as very fine powders (<0.25 mm.) in the following ratios:

	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>
Magnesite powder	4.2g.	4.2g.	4.2g.	4.2g.
Calcium carbonate	1.8g.	3.6g.	5.4g.	7.2g.
	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>
Magnesite powder	21g.	21g.	21g.	21g.
Calcium carbonate	9g.	18g.	27g.	36g.

As general manure for each pot served,

K <sub>2</sub> HPO <sub>4</sub>	0.2g.
KH <sub>2</sub> PO <sub>4</sub>	0.2g.
K <sub>2</sub> SO <sub>4</sub>	0.2g.
NH <sub>4</sub> NO <sub>3</sub>	0.8g.
Fe(OH) <sub>3</sub>	0.5g.

Oats were sown March 12, and adzuki-beans April 22, five seeds in each pot, which were diminished afterwards to three plants in the case of oats and to two in the case of adzuki-beans. Every day enough water was added to each pot to fill it to half its water holding capacity. In the first stage of development, there was hardly any noticeable difference, but it became quite marked later on, especially in the case of oats. The accompanying photographs and the following measurements date from June 18.

## LENGTH OF THE LONGEST STEM.

A <sub>1</sub>	146.6 cm.	B <sub>1</sub>	149.3 cm.
A <sub>2</sub>	170.6 "	B <sub>2</sub>	158.6 "
A <sub>3</sub>	146.6 "	B <sub>3</sub>	156.0 "
A <sub>4</sub>	147.0 "	B <sub>4</sub>	142.6 "

From this, it is clear that the lime-factor for oats is 1 irrespective of the absolute quantity of lime and magnesia offered.

The plants were cut when deadripe and weighed in the air-dry state, with the following results:

	Total harvest. (Grains and Straw.)	Roots.
	g.	g.
A <sub>1</sub>	<b>32.0</b>	4.7
A <sub>2</sub>	31.0	<b>4.8</b>
A <sub>3</sub>	30.0	4.7
A <sub>4</sub>	31.0	4.5
B <sub>1</sub>	32.0	3.4
B <sub>2</sub>	<b>35.0</b>	<b>3.8</b>
B <sub>3</sub>	33.0	3.1
B <sub>4</sub>	32.0	2.2

In the case of adzuki-beans, most leaves dropped off before all the fruits had ripened. The weights of the seeds and roots in the air-dry state were as follows:

	Seeds.	Roots.
	g.	g.
A <sub>1</sub>	2.5	1.5
A <sub>2</sub>	2.4	2.1
A <sub>3</sub>	3.6	2.1
A <sub>4</sub>	3.2	1.7
B <sub>1</sub>	2.1	0.9
B <sub>2</sub>	3.3	1.3
B <sub>3</sub>	3.5	1.7
B <sub>4</sub>	3.2	1.1

Altho the results obtained do not show very decisive differences<sup>5</sup>, the lime factor for oats is shown to lie between 0.5 and 1 and that for

5. Since it is very important to give ample room to the roots, it was found too late that three plants per pot were too many and the difference in growth was consequently less noticeable.

adzuki-beans to be about 2 whatever be the absolute quantity of lime and magnesia offered to the plants.

In the next experiments, paddy-rice and Italian millet were selected. General manure and the quantities of natural carbonates applied in each pot were quite the same as in the former experiments.

On July 16, three young rice plants were transplanted from the seed-bed into each pot. The accompanying photograph was taken on September 7. On November 5 the plants were cut and weighed in the air-dry state:

	Grains. g.	Straw. g.	Total. g.
A <sub>1</sub>	8.0	16.5	24.5
A <sub>2</sub>	14.5	15.5	30.0
A <sub>3</sub>	7.5	15.3	22.8
A <sub>4</sub>	5.7	14.8	20.5
B <sub>1</sub>	10.5	17.0	27.5
B <sub>2</sub>	10.7	17.5	28.2
B <sub>3</sub>	10.5	17.5	28.0
B <sub>4</sub>	9.5	16.0	25.5

On July 23, seven seeds of Italian millet were sown in each pot, and the plants were reduced to three of equal size later on. On Nov. 5, the plants were cut and weighed in the air-dry state:

	Fruits. gr.	Straw. gr.	Roots. gr.	Total. gr.
A <sub>1</sub>	4.2	4.5	1.6	10.3
A <sub>2</sub>	2.8	4.0	1.4	8.2
A <sub>3</sub>	2.9	3.9	1.4	8.2
A <sub>4</sub>	2.6	3.7	0.9	7.2
B <sub>1</sub>	3.8	3.8	1.2	8.8
B <sub>2</sub>	4.2	4.8	1.4	10.4
B <sub>3</sub>	3.3	3.4	0.3	7.0
B <sub>4</sub>	3.2	3.5	0.5	7.2

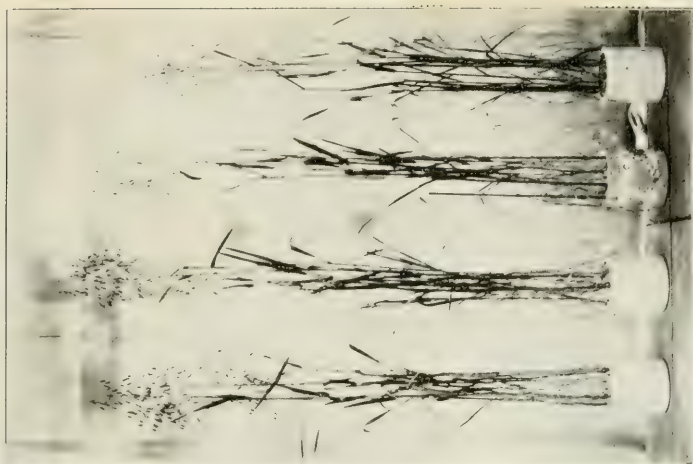
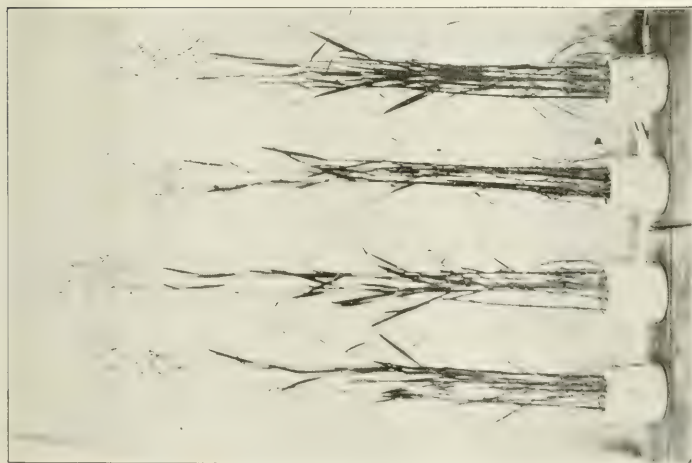
From these results, it is clear that the lime-factor for rice is 1 and that for Italian millet lies between 0.5 and 1, the absolute quantities of lime and magnesia having no influence on the development of the plants, but the ratio between these bases being the chief factor concerned.

## CONCLUSION.

1. Certain favorable ratio of lime to magnesia for plant-growth exists even in sandculture.

2. Absolute excess of lime or magnesia provided it be kept within certain limits, has no retarding effect on the development of the plants, the ratio between these bases being the chief factor for plant growth.





These plates show the influence of varying ratios of lime and magnesia on oats in sand culture.

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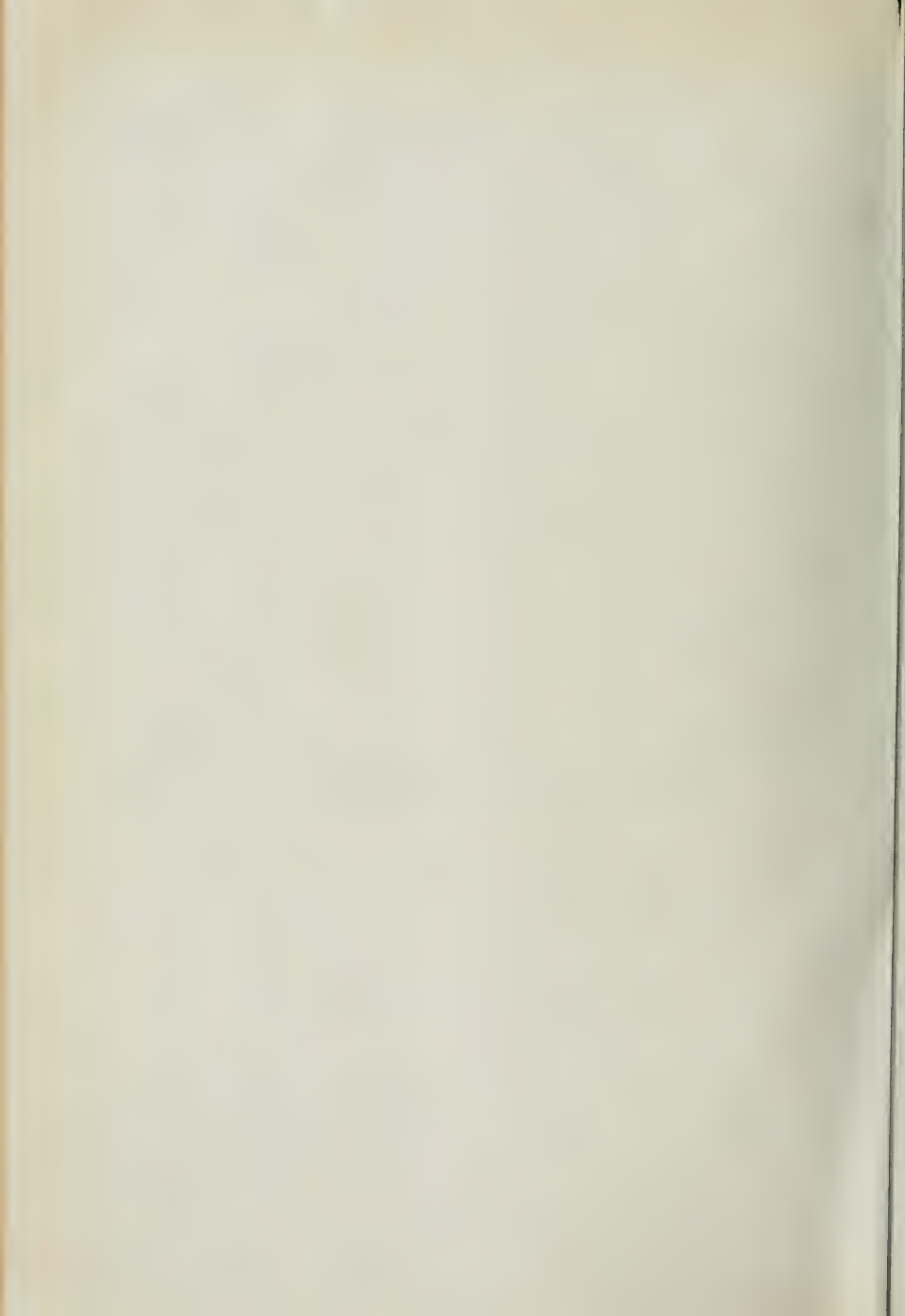


Plate V.  
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PL. IV.

Plate V.



A<sub>1</sub> B<sub>1</sub> A<sub>2</sub> B<sub>2</sub> A<sub>3</sub> B<sub>3</sub> A<sub>4</sub> B<sub>4</sub>

This plate shows the influence of varying ratios of lime and magnesia on rice in sand culture.





# Is Artificial Calcium Carbonate more Effective than Limestone Meal?

BY

H. Yokoyama.

The availability of mineral nutrients for the roots of plants depends to a large degree upon the fineness of the particles, and since precipitated compounds consist generally of finer particles than pulverised minerals it was of interest to compare the quantitative manuring effects of precipitated calcium carbonate with the finest limestone meal<sup>1</sup>. The experiment was carried out with oats in sandculture. Each pot held 2,5 kilo well purified quartzsand and received the following general manure:

$K_2SO_4$	1 g.
$(NH_4)NO_3$	0,8 "
$Na_2HPO_4$	0,5 "
$FeSO_4$	0,05 "

The magnesia was applied as magnesite meal=14g. to every pot, while the amount of precipitated calcium carbonate was varying.

Control.—Pot A received limestone meal=12 g.

B	received 3 g.	} Precipitated calcium carbonate.
C	" 6 g.	
D	" 9 g.	
E	" 12 g.	

Hereby the following ratios were produced:

1. There exists a very great difference in the manuring effects of *precipitated magnesium carbonate* and magnesite meal (see these Bulletins, vol. VII p. 609) but here the physical condition and the chemical composition also is different between the two preparations; the former is a *basic* carbonate, which is not the case with the precipitated calcium carbonate.

	A	B	C	D	E
CaO:MgO.	1:1	0.25:1	0.50:1	0.75:1	1:1

Two parallel series of pots served for the test. Six seeds of oats were sown, January 15, in each pot, and the young plants after 30 days reduced to three of equal size per pot. The plants developed well until some time after flowering when fungi commenced to show upon the leaves, whereupon the plants were cut (June 19) and weighed in the air-dry state, with the following result:

	Ratio CaO:MgO.	Number of shoots.	Total harvest, g.	Weight of grains, g. (unripe)
	Limestone meal	8	37	7.4
	1:1	11	34	6.0
Artificial calcium carbonate	0.25:1 {	7	21	4.5
		9	25	5.1
	0.50:1 {	9	26	5.4
		9	24	4.8
	0.75:1 {	10	32	5.4
		9	28	5.8
	1:1 {	12	40	7.9
		13	29	6.1

In comparing the yield of pots A and F, it will be seen that the *artificial calcium carbonate* was not essentially more effectual than *fine limestone meal*. It will be further noticed that the yield commences to sink as soon as the magnesia content exceeds the lime content in the pots, in accordance with former observations.

## On the Lime-factor for Oats.

BY

J. N. Sirker. (Calcutta.)

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The lime factor i.e. the best ratio of CaO to MgO differs considerably for different plants.

Some plants can deposit the absorbed excess of lime in the cells as calcium oxalate. Such plants can thrive well even in soils with an excess of lime.

Some plants, however, cannot thus dispose of the excess of lime and the effect shows itself in the considerable decrease of the harvest. To this group of plants belong flax<sup>1</sup> and various cereals<sup>2</sup>. These plants yield the best result when the ratio of lime to magnesia is 1, while the first mentioned group of plants thrive much better when the ratio is greater. Generally speaking, more lime is needed when the development of the leaf surface is greater in a given time.

The lime factor for cereals has been determined by several authors in soil culture. It was desirable therefore to repeat the experiment in sand culture. Quartz sand was purified with dilute hydrochloric acid and after washing well with hot water and drying, put into porcelain pots, 2½ kilos of sand to each. The following was then added to each pot as general manure:—

K <sub>2</sub> SO <sub>4</sub>	...	...	...	...	...	0.80 gr.
NH <sub>4</sub> NO <sub>3</sub>	...	...	...	...	...	0.80 gr.
Na <sub>2</sub> HPO <sub>4</sub> +12aq	...	...	...	...	...	0.50 gr.

1. Bul. College of Agric., Tokyo. VII. No. 7.

2. Cf. Bull. College of Agric. Vol. IV. No. 5, V. No. 4 and VI. No. 2.

Further, finely powdered limestone and magnesite were added in the following ratios:—

Pot.	Limestone in grms.	Magnesite in grms.	$\frac{\text{CaO}}{\text{MgO}}$
A ... ..	4.3	5.0	1.
B ... ..	8.6	5.0	2.
C ... ..	12.9	5.0	3.
D ... ..	25.8	5.0	6.
E ... ..	4.3	10.0	$\frac{1}{2}$ .

Two pots were used for each case. Ten seeds of oats were sown in each pot on the 3rd of March, 1907, and when the plants had reached the height of about 8-10 cm. they were reduced to four plants to each pot, care being taken that they were all of equal size as nearly as possible.

Water was added regularly to the pots, and they were occasionally taken out of the glass house when the weather was favourable. The number of stems in the different pots is given below:—

Pot.	A	B	C	D	E
I ... ..	16	14	13	11	12
II ... ..	14	15	11	12	14
Average ... ..	15	14.5	12	11.5	13

The ripe crop was harvested and weighed separately in the fresh state on May 22, 1907, with the following result:—

Pot.	A	B	C	D	E
I ... ..	59 gr.	58 gr.	56 gr.	53 gr.	57 gr.
II ... ..	58 gr.	51 gr.	51 gr.	49 gr.	52 gr.
Average ... ..	58.5 gr.	55.5 gr.	53.5 gr.	51 gr.	54 gr.

From this it is obvious that the lime-factor for oats in sand culture is 1, thus confirming the results obtained by previous authors with other cereals.

# On the Application of Bisulphide of Carbon in Mulberry Culture.

BY

J. N. Sirker. (Calcutta.)

Silk culture has occupied one of the most prominent places in the industrial world. Most probably China was the oldest country to produce silk. France and Italy has made a good progress in silk industry, basing the culture of the silkworm on strictly scientific principles. But in recent years Japan has introduced a new era in the silk industry of the world. In Japan, silk comes close after rice in importance as an article of domestic production, while as an article of export it has no compeer.

This important industry depends upon the rearing of the silkworm, which again depends entirely upon the cultivation of mulberry. Recently various authors have published their observations on the beneficial effect of carbon-disulphide in plant culture, when applied to the soil together with complete manures<sup>1</sup>. It seemed to me of some interest to observe the effect of the application of carbon disulphide on the growth of mulberry, and to compare it with that of an extra-dose of sodium nitrate applied as top-dressing.

Three plots, of sixteen square metres each, served for this experiment.

They were deeply and loosely ploughed, and freed from roots, stones etc. The general manure for each plot was:—

Superphosphate	...	...	...	...	...	...	20 g.
Ammonium sulphate	...	...	...	...	...	...	30 "
Calcium carbonate	...	...	...	...	...	...	20 "
Potassium sulphate	...	...	...	...	...	...	10 "
Potassium chloride	...	...	...	...	...	...	10 "

1. Although there are various opinions about the cause of the beneficial action of carbon disulphide, there is as yet no satisfactory explanation of it.

In the first plot, nine holes were made and 50 c.c. of carbon disulphide was poured into each ten days before planting. The holes were filled in immediately afterwards and water was poured on. In the second plot 40 g. sodium nitrate was applied as top-dressing in two portions—one portion on May 3, and the other on June 1, while the third plot received no special compound and served as control.

Young plants of about equal size were planted on April 5, 1907. Those of the first plot were planted in the same spots where the carbon disulphide holes were made. On September 20, the harvest was gathered with the following results:

## NUMBER OF BRANCHES.

Plot.	Plant.									Total.	Average per plant.
	1	2	3	4	5	6	7	8	9		
First ... ..	5	6	18	11	4	6	12	6	13	83	9.2
Second ... ..	6	5	11	5	5	7	4	7	4	54	6.0
Control ... ..	7	8	4	4	6	5	5	7	6	52	5.8

## HEIGHT OF THE PLANTS.

(in metres)

Plot.	Plant.									Total.	Average per plant.
	1	2	3	4	5	6	7	8	9		
First ... ..	1.70	1.69	1.43	1.55	1.75	1.53	1.82	1.62	1.51	14.60	1.622
Second ... ..	1.90	1.60	1.35	1.93	1.68	1.50	1.71	1.55	1.62	14.84	1.649
Control ... ..	1.67	1.42	1.17	1.62	1.46	1.36	1.39	1.21	1.61	12.91	1.434

## NUMBER OF LEAVES.

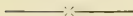
Plot.	Plant									Total.	Average per plant.
	1	2	3	4	5	6	7	8	9		
First ... ..	141	233	276	199	152	203	644	319	185	2,352	261.5
Second... ..	195	161	240	183	161	195	141	171	221	1,668	185.3
Control ... ..	250	129	143	231	157	193	135	180	215	1,633	181.4

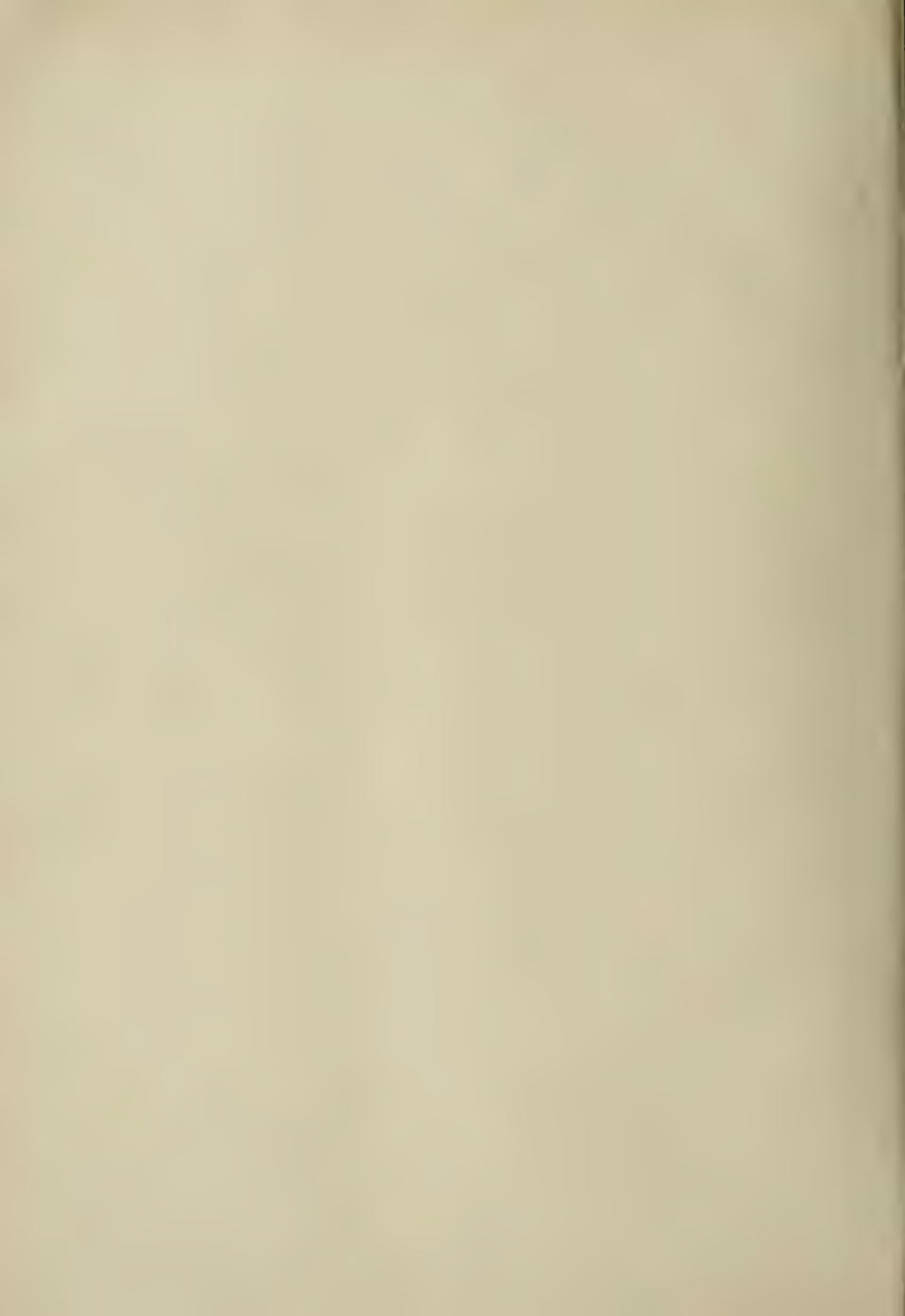
Ten leaves were selected from each plot and weighed as follows:

First plot	... ..	74.5 g.
Second plot	... ..	50.5 g.
Control	... ..	47.0 g.

This result shows that the application of bisulphide of carbon to the soil under the manuring conditions above-mentioned increased the yield of mulberry leaves by 44%, while the top-dressing with an extra-dose of nitrogen in the form of sodium nitrate was of but little use in this case<sup>2</sup>.

2. Perhaps the quantity of nitrogen applied in this case was nearly sufficient.







# Ueber die Blatt-Ernte bei *Polygonum Tinctorium* bei reichlicher Stickstoffdüngung.

VON

T. Takeuchi.

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Da reichliche Stickstoffdüngung besonders die Blattbildung befördert, und die Blätter von *Polygonum tinctorium* für Indigogewinnung in Japan trotz der bedeutenden Einfuhr künstlichen Indigos noch eine grosse Rolle spielen, war es von Interesse, zu beobachten, wie weit die Blattbildung bei dieser Pflanze durch reichliche Stickstoffdüngung befördert werden könnte.

Es wurden vier Parzellen zu je 12 Quadrat-metre auf einem lehmigen Humusboden zunächst mit dem gleichen Grunddünger, nämlich 2400 g. Kompost (=2000 kilo pro ha.) + 300 g. Holzasche (= 250 kilo pro ha.) versehen. Die Parzellen A und C dienten zur Kontrolle, während B. u. D. zweimal je 180 g. Chilisalpeter als Kopfdüngung erhielten. Die jungen Pflanzen wurden am 21 Juni eingesetzt, die Entwicklung fand ohne jede Störungen statt.

Die Ernte am 13 August ergab.: Gewicht, lufttrocknen, g.

	A	B	C	D
Blätter ... ..	650	1160	770	1255
Stämme ... ..	1435	1365	1410	1550

Nun wurde nach dem Schneiden den jungen Trieben freie Entwicklung gelassen, um eine zweite Ernte zu erzielen. Die Parzelle D erhielt diesmal dieselbe Dosis Nitrat wie das erste Mal, während B nur 200 g. Nitrat als Kopfdüngung in 2 Dosen. Die eine Kontrollparzelle C aber erhielt diesmal 400 c.c. Schwefelkohlenstoff, welcher in kleinen Dosen

in etwa  $1\frac{1}{2}$  Fuss tiefe Löcher zwischen die Pflanzen eingegossen wurde. Am 9 October wurde die Pflanzen geschnitten mit folgendem Resultat: Gewicht, lufttrocken, g.

	A	B	C	D
Blätter ... ..	630	785	760	823
Halme ... ..	895	960	955	990

Die vermehrte Stickstoffdüngung hatte also sehr günstig auf den Blattertrag gewirkt, da der Mehrertrag an Blättern (erste und zweite Ernte zusammen) bei B=52% und bei D=62% über A betrug. Die Schwefelkohlenstoffbehandlung hat mehr auf Stengel als auf Blattbildung gewirkt.

Auf denselben Parzellen wurde im folgenden Jahre noch ein Versuch mit etwas abgeänderter Grunddüngung ausgeführt:

Grunddüngung { 3 kilo Kompost pro 12□m. (=2500 kilo pro ha.)  
+ 120 g. Holzasche pro 12□m. (=100 kilo pro ha.)

D erhielt einige Zeit vor der Pflanzung 400 c.c. Schwefelkohlenstoff. Die Kopfdüngung war:

A ... ..	Kontrol, keine Kopfdüngung.
B ... ..	$N_4NO_3$ 300 g. in zwei Fraktionen.
C ... ..	360 g. „ „ „

Die Ernte am 24 August ergab.: Gewicht, lufttrocken, g.

	A	B	C	D
Blätter ... ..	1170	1325	1665	1285
Halme ... ..	1680	1695	2710	1890
Total ... ..	2850	3020	4375	3175

Nach dem Schneiden wurde den Trieben freie Entwicklung gelassen. Diese zweite Ernte, am 7 October im lufttrocknen Zustande gewogen, betrug, g.:

	A	B	C	D
Blätter ... ..	413	514	572	535
Halme ... ..	306	453	510	436
Total ... ..	719	967	1082	971

Setzen wir die Ernte an Blättern auf der

Kontrollparcelle  $A=100$ , so ist der Ertrag

bei  $B=116.1$

„  $C=141.3$

„  $D=114.3$

Die Schwefelkohlenstoffbehandlung hat somit einen geringeren Mehrertrag gebracht als reichliche Stickstoffzufuhr in Form von Kopfdüngung mit Chilisalpeter.



# On the Application of Dicyandiamide as a Nitrogenous Manure.

BY

R. Inouye.

Since manuring with '*lime-nitrogen*' will gradually spread according to its importance for agriculture, it will be of some interest to investigate the suitable method of the application of dicyandiamide as a manure. For this purpose the following experiments were made.

Five pots containing 8 kilo air dry soil were manured as follows:

Pots.	Superphosphate.	Potassium carbonate.	Ammonium sulphate.	Dicyandi- amide.
I. (Control)	10 g.	5 g.	5 g.	0
II.	10	5	3.3	0.75 g.
III.	10	5	3.3	0.75 (as topdressing)
IV.	10	5	0	2.2
V.	10	5	0	0

Ammonium sulphate employed contained 21% nitrogen, and dicyandamide<sup>1</sup>, being somewhat impure, 46.7% nitrogen.

As the above table shows, the quantities of nitrogen applied were equal in each pots, except the pot V, which contained no nitrogenous manure at all. Thus the pot I served as control, and contained the total nitrogen as ammonium sulphate; the pot II,  $\frac{2}{3}$  of the total nitrogen as ammonium sulphate and the rest  $\frac{1}{3}$  of the nitrogen as dicyandiamide, while the pot III received the manure in the same porportion as the pot II, but dicyandiamide being given as top-dressing later on; the pot IV, the total nitrogen as dicyandiamide.

1. The writer prepared this compound from '*lime nitrogen*.'

For the experimental plants served rape and barley.

On November 26, twenty seeds of each plant were sown in each pot after selecting healthy ones by steeping them into water.

(1) *Experiment with Rape.*

On January 17, the young plants in each pot were reduced in number to ten of nearly equal size. The plants in the pot I, II, and III have grown well, especially the last two very well. Eight weeks after germination, the margins of the leaves of the plants in the pot IV became yellowish white showing the injurious effect of dicyandiamide and their growth was very much retarded. On February 22, they were again reduced to five. Although at the beginning of the growth the plants in the pot II were injured, they were quite recovered after four months, and grew so well as the control plants. On Feb. 28, 0.75 g. of dicyandiamide dissolved in one liter of water was topdressed to the pot III. After three weeks the margin of the leaves of the rapes in this pot began to wither, but the damage was not so severe as in the pot IV.

By this time, the plants in the pot IV have shown the same unfavorable emen like those in the pot V.

On March 12, almost all of the plants came to bloom, when the accompanying photograph was taken (see the plate I).

The crop was harvested and weighed in the fresh state on April 14. The following results were obtained.

AN AVERAGE WEIGHT FOR ONE PLANT.

Pots.	Weight of the upper part of plants above roots.	Weight of roots.	Weight of whole plant.
I.	57	2.4	59.4
II.	60	2.6	62.6
III.	61	3	64
IV.	7.8	0.6	8.4
V.	5	0.4	5.4

(2) *Experiment with Barley.*

The seeds were sown on November 26. On January 17, they were reduced to ten of equal size per pot. In the early period of the growth, no particular phenomena were observed, but after eight weeks since they had been sown, the leaves of the plants in the pot IV, which received 2.2 g. of dicyandiamide, began to wither from their tips and edges, and these withered parts gradually developed. The injurious action of the manure was also observed on the plants in the pot II, but it was not so severe as in the pot IV. On February 22, when the young plants had reached to 11-15 cm. high, they were again reduced to seven. On Feb. 28 the pot III received a new dose of 0.75 g. dicyandiamide, dissolved in one liter of water. Although the plants in pot were injured to a certain degree, they began to show the most favorable growth among all after three weeks. In the middle of April, the beneficial effects of dicyandiamide became gradually more noticeable, and the plants in the pot III were the best; the plants in the pot II next, and those in the control pot third, while the growth of those in the fourth pot was not so much retarded as that of the rapes in the fourth pot. Since some of the plants were damaged by fungi, they were reduced to four per pot on April 30. The accompanying photograph (the plate II.) was taken on May 14, when they came to full growth.

The plants were harvested on June 19, and weighed in the air-dry state with the following results.

AVERAGE WEIGHT FOR ONE PLANT.

Pots.	Number of branches.	Weight of dry plants (roots taken off.)	Weight of straws.	Weight of ears with grains.	Weight of grains.
I.	3	8.3	4.8	3.5	2.7
II.	3	9	5	4	3.1
III.	4	9	5	4	3.5
IV.	2	2.5	1.5	1.2	1
V.	1	1.8	1	0.8	0.7

## CONCLUSION.

Dicyandiamide may be injurious to the plants, but if the dose does not surpass a certain limit, it does rather act beneficially to them, and it is, indeed, a better manure than ammonium sulphate.

The pot I, II, III and IV received an equivalent quantity of nitrogen in the different forms, but the plants in the pot III to which dicyandiamide was topdressed, yielded the best harvest of all, on the contrary, the plants in the pot IV, which received dicyandiamide as a only nitrogenous manure, were as much injured as those in the pot V which received no nitrogen.

From these results, we can say that one gram of nitrogen in the form of dicyandiamide for eight kilo soil is even injurious to the crops and especially to the young plants, while 0.35 g. of nitrogen in the same form yields a favorable result. It may be inferred that if dicyandiamide is applied as a manure, it is better to use it as topdressing.



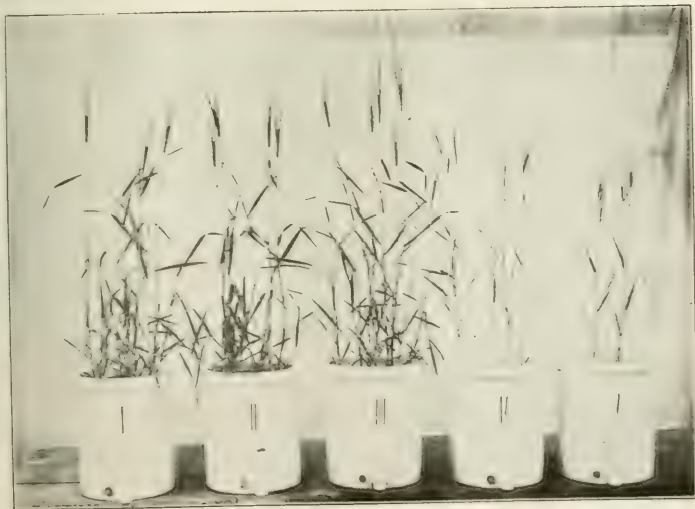
Pl. I.

Plate VI.

21.691  
1955



Pl. II.





## Some Improvements in Sand Culture.

BY

T. Takeuchi.

---

Various methods of manuring sandcultures have been proposed to overcome certain difficulties. Since sand has no absorptive power, the reaction of the manure salts and the concentration of the "soil solution" is here of much greater importance than in soil culture. The quick evaporation of moisture from sand adds to the complication. In some cases it was proposed to apply phosphoric acid as monopotassium phosphate, which, however, on account of its acidity may exert a certain injurious action owing to the rapid evaporation of the water and the consequent concentration of the solution. The guiding principles for obtaining favorable results in sand culture must be:

- (1) The diminution of the amount of soluble salts,
- (2) The insoluble ingredients of the manure must be very easily available for the roots,
- (3) Care must be taken to preserve a neutral reaction as far as possible during the time of vegetation.

These considerations have led the writer to apply nitrogen in the form of ammonium nitrate<sup>1</sup>; further phosphoric acid in the form of a mixture of mono- and dipotassium phosphate to ensure neutral reaction. Lime and magnesia were applied as finely powdered natural carbonates in a favorable ratio for the common cereals. Further, a small amount of

1. Hellriegel (Ueber das Stickstoffbedarf der Gerste) says: "Ammonium sulphate and chilisaipetre are unfit for sandculture." Since an acid, or alkaline reaction, will be gradually produced by these compounds, this statement appears justified.

gypsum was added, not only as a source of the necessary sulphur, but also for neutralising any alkaline reaction that might gradually result. Finally a small dose of sodium chloride was added, since the favorable action of sodium as well as of chlorides is well established. The iron was applied as ferric hydroxyde.

It was further desirable to institute a comparison to Hellriegel's sandculture, who<sup>2</sup> used for 4 kilo sand:

$\text{KH}_2\text{PO}_4$	...	...	...	...	0.4083 g.
KCl	...	...	...	...	0.1492 "
$\text{MgSO}_4$	...	...	...	...	0.1800 "

Further calcium as carbonate 4 g., while the addition of calcium nitrate<sup>3</sup> was varied in order to observe the effect of different doses of nitrogen.

The pots of the writer contained 4 kilo quartz sand to which was added,

$\text{K}_2\text{H}_2\text{PO}_4$	...	...	...	...	0.3 g.
$\text{K}_2\text{H}_2\text{PO}_4$	...	...	...	...	0.3 "
$\text{K}_2\text{SO}_4$	...	...	...	...	0.3 "
$\text{NH}_4\text{NO}_3$	...	...	...	...	1.2 "
NaCl	...	...	...	...	0.3 "
$\text{CaSO}_4$	...	...	...	...	0.6 "
$\text{Fe}(\text{OH})_3$	...	...	...	...	0.8 "
Magnesite	} 4	...	...	...	6.7 "
Limestone		...	...	...	5.3 "

2. It seems strange that Hellriegel in two publications, namely on the "Stickstoffbedarf der Gerste" and on the "Assimilation of free nitrogen by Leguminosae" do not mention in what form the iron was applied in his sandcultures. In his "Grunddüngung" the addition of iron compounds is not mentioned! The writer has, nevertheless, added iron in his pots as ferric hydroxide.

3. In this case 1.68g was used.

4. Both these carbonates were in the form of very fine powder < 0.25 m.m.

To a second pot (K) 5% kaolin<sup>5</sup> was added in order to produce some absorptive power and to increase the water retaining capacity. A third pot (H) was prepared exactly according to the prescription of Hellriegel. The water was so regulated that it corresponded to 15% of the sand, i.e. 60% of the water holding capacity of the sand. In other words the 4 kilo of sand was so irrigated that it contained 600 g. water. Thus the concentration of the nutrient solution would be 0.4%. But to avoid this somewhat high concentration,<sup>6</sup> the ammonium nitrate was applied in two doses one half before sowing and the other half when the young plants had reached the height of about 20 cm.

The plants used were upland rice, barley, wheat and oats.

### *Experiment with Upland Rice.*

8 seeds were sown April 21 and the young plants reduced later on to 4 per pot, all of nearly equal size. At first, the pot (H) seemed rather prosperous, but after the application of the second dose of ammonium nitrate this condition was reversed, (I) and (K) showing more rapid progress. The plants were cut Nov. 4 with the following results (air dry).

	Number of		height (average)	Weight of		
	cars	grains		grains	straw	total
K	4	288	90 cm.	8.9 g.	14.6 g.	23.5 g.
I	5	297	87 „	8.1 „	12.6 „	20.7 „
H	4	107	69 „	3.3 „	7.1 „	10.4 „

5. This kaolin contained but few impurities and was of neutral reaction. 100 g. treated with HCl of 10% yielded after evaporation of this extract only 0.63 g. residue which contained no phosphoric acid, but only a small quantity of iron and potash. A further examination of the kaolin yielded:

FeO	...	...	...	...	0.43 %
K <sub>2</sub> O	...	...	...	...	0.12 „
MgO	...	...	...	...	0.04 „
CaO	...	...	...	...	0.01 „

6. Hellriegel (Grundlagen des Ackerbaues, p. 275) considers 9.2 ‰ concentration of the solution in the sand as not injurious for the young plants, but the writer has avoided this concentration.

*Experiment with Oats.*

The experiment was carried out just in the same manner as in upland rice, with three pots in the glass house; but the pots contained three kilo purified quartz sand instead of four kilo. The manure of (I), therefore, consisted of

$K_2H_2PO_4$	...	...	...	...	0.225 g.
$K_2H_2PO_4$	...	...	...	...	0.225 "
$K_2SO_4$	...	...	...	...	0.225 "
$NH_4NO_3$	...	...	...	...	0.900 "
NaCl	...	...	...	...	0.225 "
$CaSO_4$	...	...	...	...	0.450 "
$Fe_2H_2$	...	...	...	...	0.003 "
Magnesite	...	...	...	...	5.000 "
Li-stone	...	...	...	...	1.000 "

(K) and (II) also received similar treatment.

8 seeds were sown Dec. 9 and reduced later on (Jan. 25) to 3 per pot, all of nearly equal size. The second dose of ammonium nitrate was applied March 7. A difference in development soon became evident after this and on the 22 of April the plants in (I) and (K) showed ears, almost at the same time, while those in (II) took ten days longer. The appended photograph was taken at this stage. The measurement on May 10 was as follow:

	Number of ears.	Average height.
K	9	118 cm.
I	10	105 "
II	7	86 "

The plants were cut June 16 and weighed in the air dry state, with the following results:

	Length of		Number of			Weight of			
	stalk	roots	ripened seeds	unripened seeds	ears	ripened seeds	ears	straw	roots
K	123 cm.	24.6 cm.	238	114	9	9.01 g.	15.6 g.	43.4 g.	9.62 g.
I	114 "	22.8 "	186	135	10	5.49 "	10.7 "	45.7 "	8.14 "
II	108 "	17.5 "	101	86	7	3.59 "	6.5 "	42.0 "	7.35 "

*Experiments with Barley and Wheat.*

These experiments were made exactly in the same way as in the preceding case. Sowing, reduction, topdressing and harvesting were also carried out as in the preceding experiment. The harvests were weighed June 16 in the air dry state with the following results:

## BARLEY.

	Length of		Number of		Weight of				
	stalks	roots	ripened seeds	ears	ripened seeds	ears	straw	roots	total
K	87.7 cm.	24.3 cm.	136	11	7.89 g.	12.8 g.	25.3 g.	5.4 g.	43.5 g.
I	83.6 „	14.5 „	193	11	8.78 „	13.5 „	17.4 „	3.8 „	34.7 „
II	68.9 „	12.9 „	66	7	3.25 „	6.8 „	14.1 „	3.3 „	24.2 „

## WHEAT.

	Length of		Number of		Weight of				
	stalks	roots	ripened seeds	ears	ripened seeds	ears	straw	roots	total
K	118 cm.	27.2 cm.	341	10	11.35 g.	15.3 g.	28.9 g.	8.61 g.	52.81 g.
I	103 „	25.5 „	386	13	10.61 „	14.9 „	26.5 „	7.18 „	48.58 „
II	105 „	21.3 „	217	9	9.05 „	11.3 „	26.0 „	5.64 „	42.94 „

These results show that Hellriegel's prescription for sandculture can be improved by the modification here proposed. The addition of kaolin (5%), however, has to be avoided in cases in which *potassium compounds* come in question. When the question relates to nitrogen or phosphoric acid, its addition can only be of benefit and not misleading. The amount of limestone should be increased for other plants than the Gramineæ and flax. The mixture of the author for Gramineæ in sandculture is:

Sand	...	...	...	...	...	4000.0 g.
$K_2H_4PO_4$	...	...	...	...	...	0.3 „
$K_1H_2PO_4$	...	...	...	...	...	0.3 „
$K_2SO_4$	...	...	...	...	...	0.3 „
$NH_4NO_3$	...	...	...	...	...	1.2 „ (to be applied in 2 fractions)
NaCl	...	...	...	...	...	0.3 „

$\text{CaSO}_4$	...	...	...	...	...	...	0.6 g.
Magnesite	...	...	...	...	...	...	6.7 "
Limestone	...	...	...	...	...	...	5.3 "
$\text{Fe}(\text{OH})_3$	...	...	...	...	...	...	0.8 "

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2021

PL. I.

Plate VII.

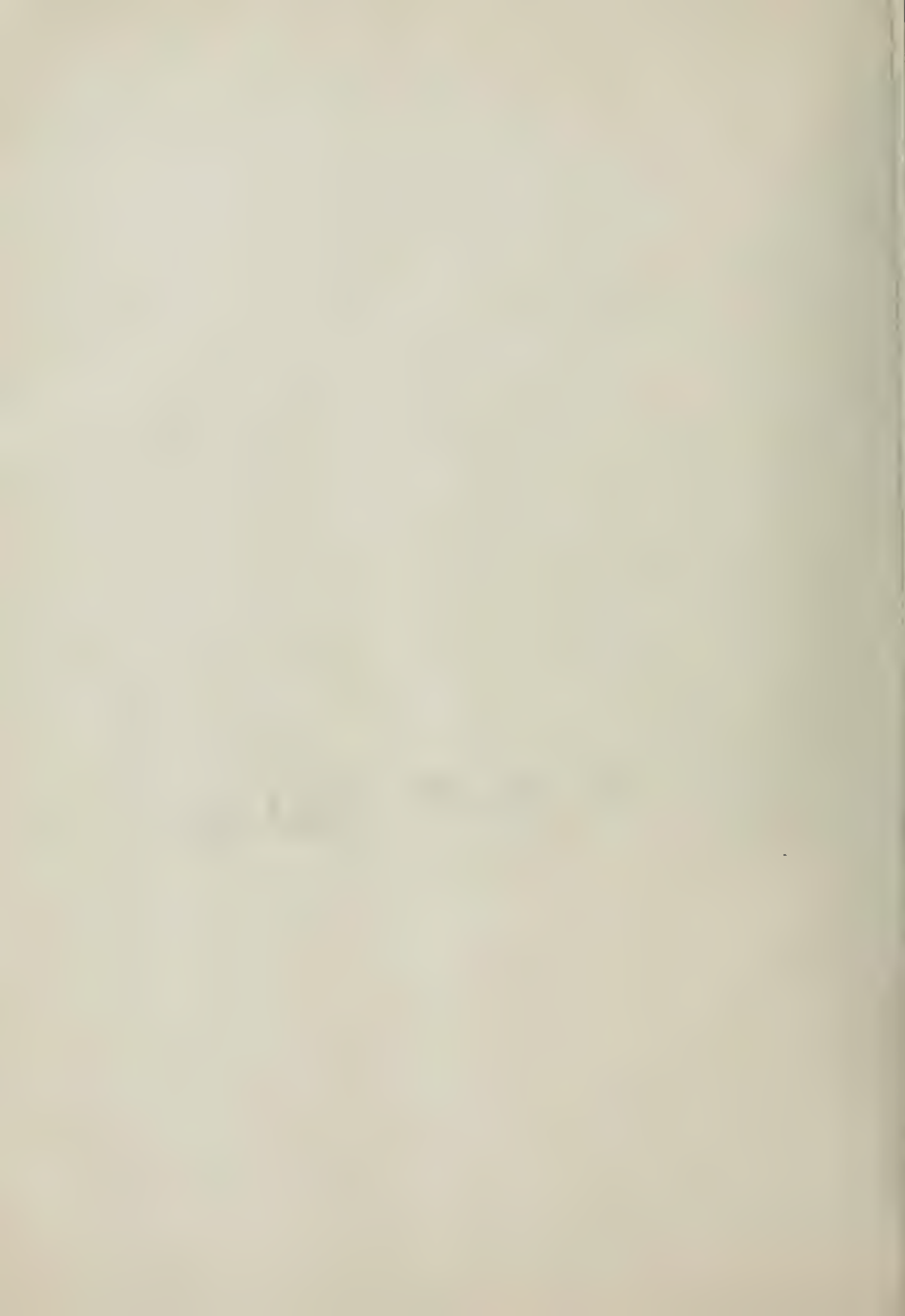




PL. II.

Plate VIII.





PL. III.

Plate IX.



K

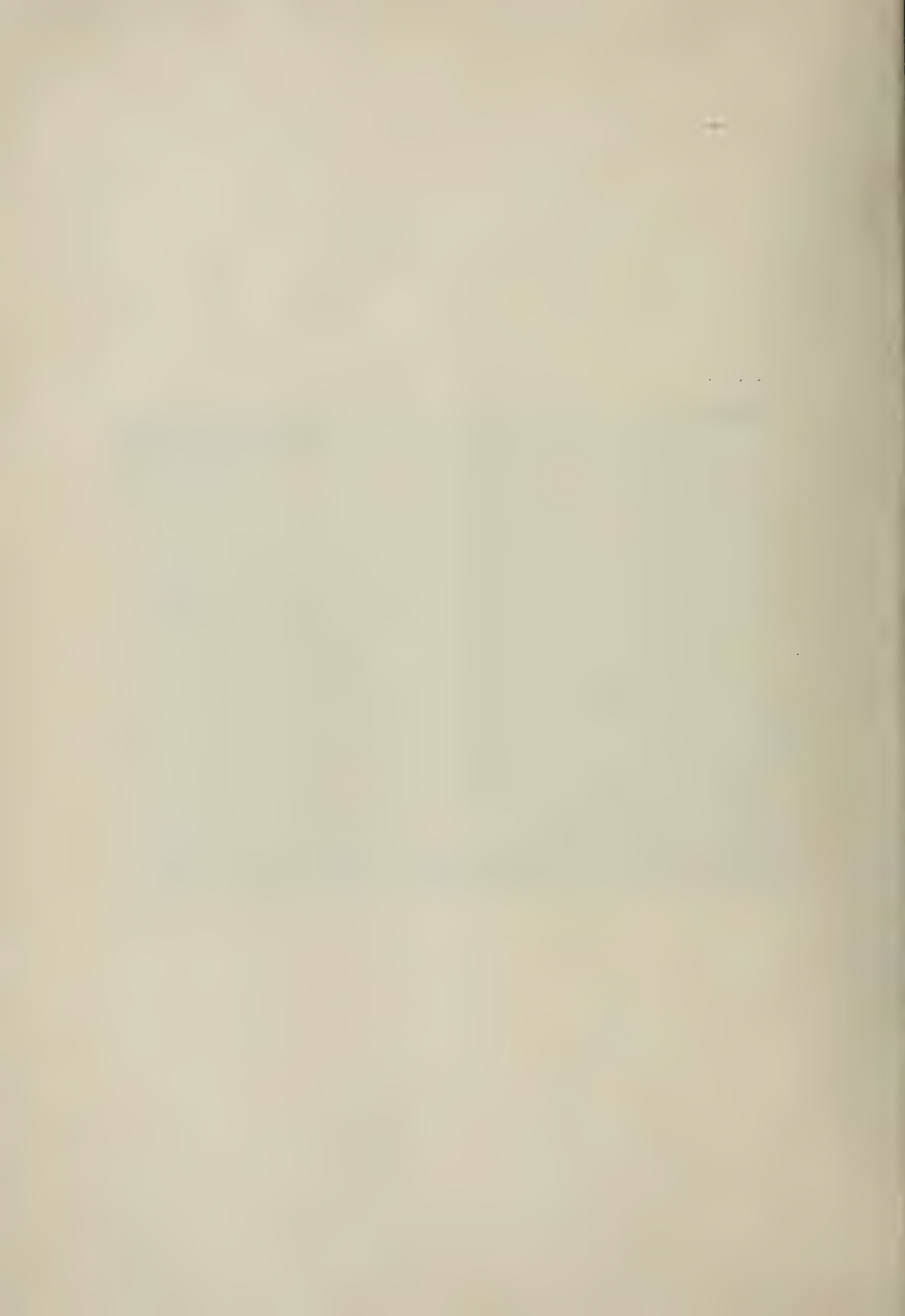
I

II

K

I

II



## Secondary Calcium Phosphate as a Manure.

BY

T. Takeuchi.

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Repeated experiments carried out at this college have shown that the most favorable ratio lime to magnesia for the smaller Gramineæ lies between 1:1 and 2:1. It remained to be tested, in how far under this condition the harvest is influenced by varying amounts of phosphoric acid, when these three substances are present in the soil in about an equal state of availability. According to the theory there ought to exist a considerable influence, when the ratio of phosphoric acid to the limefactor differs widely in the cells. And that there exist often varying ratios is shown by the ash analyses of the same plants grown on different soils. Further, the analyses of soils show, that the amounts of  $P_2O_5$  are generally smaller than those of lime and magnesia combined, while frequently the former surpass either those of lime or of magnesia separately.

In my experiments lime and magnesia were applied as natural carbonate in a very finely powdered condition ( $< 0.25$  m.m.), while phosphoric acid as precipitated secondary calcium phosphate, since these compounds were supposed to have about an equal state of availability. Lime, magnesia and phosphoric acid were applied in sandculture in the following proportions;

$$(A) \frac{CaO}{MgO} : P_2O_5 = \frac{1}{1} : 1 ; \quad (3 \text{ g. each})$$

$$(B) \frac{CaO}{MgO} : P_2O_5 = \frac{5}{5} : 1 ;$$

$$(C) \frac{CaO}{MgO} : P_2O_5 = \frac{1}{1} : 5 ;$$

$$(D) \frac{CaO}{MgO} : P_2O_5 = \frac{5}{5} : 5.$$

From these ratios the amounts of the above compounds were as follows:

	CaCO <sub>3</sub> , g.	MgCO <sub>3</sub> , g.	CaHPO <sub>4</sub> + 2aq., g.
(A)	5.36	6.25	3.63
(B)	26.78	31.33	3.63
(C)	5.36	9.25	18.18 <sup>1</sup>
(D)	26.78	31.33	18.18

Four pots each holding 3 kilo well purified sand, received the following general manure, each:

K <sub>2</sub> SO <sub>4</sub>	0.5 g.
NH <sub>4</sub> .NO <sub>3</sub>	1.0 "
Fe (OH) <sub>3</sub>	0.25 "
NaCl	0.05 "

The ammonium nitrate was applied in 2 portions, the second half as topdressing, when the young plants had reached 20 cm in height (July 9), the first half before sowing.

As crop served upland rice of which 8 seeds were sown in each pot (May, 20); the young plants when 15 cm. high (June 25) were reduced to 4 per pot, all of nearly equal size. During the vegetation no disturbance occurred. At the end of August the plants in A and C developed ears, those in D 22 days later. The plants in B never showed ears at all. The plants were harvested Oct. 2, with the following results, air dry, g.

	Average ht.	Total wt.	Number of ears.	Number of grains.	Wt. of grains
A	74 cm	7.53	4	108	2.0
B	68 "	5.22	No ear	No grains	0
C	75 "	9.78	5	151	3.3
D	70 "	7.65	3	41	0.95

This result shows that a great excess of carbonates of lime and magnesia can depress the absorption of phosphoric acid from secondary

1. By this increase the ratio CaO : MgO was considerably changed.



calcium phosphate so much, that the formation of ears (with rice) becomes impossible. This depression of harvest can only partially be overcome by raising the amount of this phosphate to the amount of calcium carbonate present as the comparison of the harvests in B and D. will show. By this increase of secondary calcium phosphate, however, also the most favorable ratio of lime to magnesia in the sand was altered somewhat. In comparing harvests A and C it will be noticed that by changing the ratio  $\text{CaO}:\text{MgO}:\text{P}_2\text{O}_5 = 1:1:1$  into  $1:1:5$  an increase of the total harvest of 29.9% and of grains of 65% has resulted. Hence the increase of phosphoric acid in the form of secondary calcium phosphate has exerted such a favorable effect that the depression resulting by the great increase of lime taking place by manuring with secondary calcium phosphate and thereby changing the most favorable original ratio of  $\text{CaO}:\text{MgO}$ , became not noticeable. In comparing the harvest A and B, the detrimental effect can be noticed, which is experienced, when soils containing phosphatic manures in a water insoluble state are limed in a high degree.

Under certain conditions (probably when no or but little carbonates are present in soils) the secondary calcium phosphate is an excellent phosphatic manure, as Prianischnikow<sup>1</sup> has shown.

It will appear to us, that although secondary calcium phosphate is less soluble in dilute acids as calcium or magnesium carbonate so small amounts of carbonates, as can depress the availability of *tertiary* calcium phosphate, exert still but little effect on that of *secondary* calcium phosphate and that this requires larger doses for the depression of its availability.

As to superphosphate, however, probably only very large doses of carbonates can lead to a depression of its availability. This inference may be permitted by varying a result obtained by Hamasaki<sup>2</sup>. In his experiment disodium phosphate served as manure. In this case the change

2. Landw. Versuchs-Stationen. 56, p. 122.

3. These Bulletins, Vol. VII, p. 607.

of the ratio  $\text{CaO}:\text{MgO} = 1:1$  to ten times the amount (phosphoric acid being constant) the harvest showed a moderate depression of 14%.<sup>3</sup>

The question will entirely change its aspect, when lime, magnesia and phosphoric acid are applied in *water soluble* forms, or when lime and magnesia are present in soils *not in the form of carbonates*. Under these conditions the varying ratios between the lime factor and phosphoric acid will doubtless show other and very interesting results. I hope to continue these investigations.

4. Cf. Also Westhauser and Zielsdorf, C. f. Agric. Chem., 1908.

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## On Differences of Susceptibility of Plants to Stimulation.

BY

T. Takeuchi.

Since it had been observed here at this College on several occasions that certain plant species are more susceptible of stimulation than others, it seemed desirable to extend such observations.<sup>1</sup>

The following plants were compared under equal circumstances; spinach, pea, barley and flax.

8 pots each holding 8 kilo of a loamy soil received the following general manure:

8 g.	Double superphosphate
5 „	Potassium sulphate
10 „	Sodium nitrate
5 „	Ammonium sulphate

Four pots served as control pots, while of four pots each received 0.2 g. crystallized managanous sulphate  $MnSO_4$  + 4 aq. which was incorporated in the form of a very dilute solution (1 p. mille), while the control pots received an equal amount of water.<sup>2</sup>

1. It may here be mentioned that according to *Körnike* the seeds of *Vicia faba* are much more sensitive to radium rays than the seeds of *Brassica*.

2. A former similar experiment with 2 g. manganous sulphate per pot had shown that this dose was too large and somewhat injurious, especially for the young plants. These results were: g.

	Barley		Pea		Flax		Spinach	
	Control	Mn.	Control	Mn.	Control	Mn.	Control	Mn.
Total Wt.	109.5	106.4	102.4	103.2	50	47	140	82
Seeds	48.1	47.7	56.2	55.1	—	—	—	—
Fruits	—	—	—	—	27	26	—	—

Mr. Muramatsu had observed further that topdressing with 1 g.  $MuSO_4$  for 10 kilo soil depressed the yield also; these doses are therefore too large.

*Spinach.*

Twenty seeds were sown Nov. 10. The number of the young plants when 3-4 cm. high was reduced to 8 per pot all of nearly equal height. The plants showed gradually a considerable difference in development:

		Average height :	
		Control	Manganese
Dec.	19	4 cm.	4.5 cm.
Jan.	29	6 "	10.1 "
Mar.	2	22 "	26.0 "

The plants were cut March 22; the weight in the fresh state was:

Control plants	129 g.
Manganese plants	182 "
Hence increase = 41%	

*Pea.*

Twenty seeds per pot were sown Nov. 10 and the young plants thinned after two weeks to 8 in each pot.

		Average height :	
		Control	Manganese
Dec.	19	7.2 cm.	9.3 cm.
Jan.	29	13.6 "	16.0 "
Mar.	1	27.5 "	29.4 "
April.	1	56.4 "	62.6 "

The plants were cut May 24 and left to dry; the air dry weight was:

	Total	Pods	Seeds	100 seeds
Control	71.5	46.8	34.3	27.3
Mn.	85.4	48.2	26.1	29.2

Hence total harvest increase = 19.4% which is about one half of the increase, observed in the previous experiment.

*Barley.*

Twenty seeds of Barley were sown Nov. 10 and the young plants later on thinned to 10 of nearly equal size:

		Average height :	
		Control	Manganese
Dec.	19	14.2 cm.	15.1 cm.
Jan.	29	18.5 "	19.4 "
Mar.	1	28.5 "	28.8 "
Apr.	1	42.4 "	43.9 "

The plants were cut May 28 and weighed in the air dry state with the following results, g:

	Total	Grains	Straw
Control plants	140.8	51.0	89.8
Manganese plants	148.3	50.4	97.9

Hence increase of total harvest = 5.3%

#### *Flax.*

Twenty seeds were sown Nov. 10 and later on the young plants reduced to 8 per pot, all of nearly equal size. The growth and flowering of the control plants was behind that of the manganese plants:

		Average height :	
		Control	Manganese
Dec.	19	3.3 cm.	4.0 cm.
Jan.	29	5.7 "	6.4 "
Mar.	1	10.1 "	12.0 "
Apr.	1	18.2 "	21.0 "

The plants were harvested June 11 and gave the following results:

	Total weight	Weight of fruits	Number of fruits.
Control plants	32.3 g.	11.3 g.	169
Manganese plants	36.8 g.	13.2 g.	187

Hence increase of total harvest = 13.9%

This result shows indeed that different plant species are not stimulated at an equal degree by manganese, under the same conditions. Former observations at this College seemed to prove that Leguminosæ and Cruci-

feræ were more susceptible than Gramineæ. Also this time a Graminea, viz. barley, was the least stimulated.<sup>3</sup> The comparison shows:

Percent of Stimulation.						
Barley ... ..	...	...	...	...	...	5.3%
Flax ... ..	...	...	...	...	...	13.9 „
Pea ... ..	...	...	...	...	...	16.4 „
Spinach ... ..	...	...	...	...	...	41.0 „

3. The absorbed manganese passes in the Gramineæ probably soon into an insoluble form.

## On Manuring with Dicyandiamid.

BY

K. Asō.

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Numerous experiments with calcium cyanamid showed that in many cases this nitrogenous manure was equal or nearly so to sodium nitrate or ammonium sulphate.<sup>1</sup> On acid muck soils and on sandy soils, however, less favorable results have been obtained.<sup>2</sup> Acid soils should at first be neutralized with lime before lime-nitrogen is applied. Further it is advised to avoid mixing of lime-nitrogen with superphosphate.<sup>3</sup>

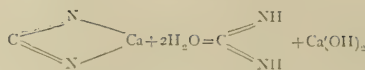
Lime-nitrogen is at present manufactured with addition of calcium chlorid, since under this condition the process goes on at a less high temperature. But this product gradually attracts moisture and loses nitrogen in form of ammonia. Hence Carlson<sup>4</sup> proposes to replace the calcium chlorid by calcium fluorid. There exist however circumstances under which lime-nitrogen would not act as favorably as expected, especially, when the soils are not only rich in lime, but also when they contain much more lime than magnesia, as by the application of this lime compound the lime content of the soil will be unduely increased. Hence it had been proposed some years ago to separate the lime from the cyanamid. When the crude lime-nitrogen is treated with warm water, a separation takes place into cyanamid and calcium hydroxid:

1. See also this bulletin. Vol. IV. No. 1. Also Tmmendorf: Fühlings lowdw.-Ztg. 1905. S. 794.

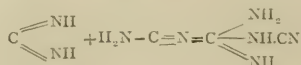
2. Lawdw. Presse. 16. Jan. 1907.

3. It is probable that acid liquid prevent the change of the poisonous cyanamid into the not poisonous dicyandiamid.

4. Chem. Zg. 1906. Nr. 101.



and the cyanamid thus formed readily polymerizes to dieyandiamid:



According to Ulpiani, this dieyandiamid is not poisonous while cyanamid is poisonous. Perotti<sup>5</sup> found that higher plants are only injured by solutions containing three per thousand or more dieyandiamid, different plants having varying powers of resisting its action and lower organisms (algae and bacteria) are more resistant than higher plants. He concluded that calcium cyanamid is far more injurious than dieyandiamid and that the manurial value of calcium cyanamid probably depends on its conversion into dieyandiamid. My own experiments have convinced me that indeed dieyandiamid is perfectly harmless, for even at the concentration of 1%, it did not injure algae as *spirogyra* at least within five days,<sup>6</sup> certainly a most surprising fact. Later on the writer observed that dieyandiamid can not well be utilised by certain soil bacteria, but cells of higher plants as a source of nitrogen at the concentration of 0.1%. Ulpiani and Perotti had used mainly culture solutions, but Wagner and Immendorf observed in potcultures with various soils a decidedly poisonous action of dieyandiamid. This apparent contradiction was cleared up by Loew,<sup>7</sup> who observed a poisonous action of dieyandiamid on a common soil, while in contrary a very favourable result with sterilised soil. Hence the inference was drawn that certain microbes in the soil produce some poisonous compound from dieyandiamid.

5. Journ. of chem. Soc., April 1907.

6. This non-poisonous character renders the imido formula  $\text{NH}=\text{C} \begin{array}{c} \diagup \quad \diagdown \\ \text{NH} \quad \text{NH} \end{array}$  for dieyandiamid more probable than the cyanoguanidin-formula, as neither a cyanogengroup nor an amidogroup would be present.

7. Chem. Ztg. 1909, Nr. 3.



It was of interest, however, to make further experiments along this direction.

In the first place, my own experiment with bacteria in pure culture have shown that neither *Bacillus mycoides* nor *Bacillus fluorescens liquifaciens* nor *Bacillus subtilis* can utilise dicyandiamid as a favorable source of nitrogen, the development even after three weeks consisting only a turbidity of the liquid. The culture solution I applied had the following composition.<sup>8</sup>

0.1 %	dicyandiamid.
0.5 %	mannitol.
0.2 %	$K_2HPO_4$
0.02%	Mg $SO_4$

It is to be regretted that Perotti did not given a detailed characteristic of his bacteria which could utilize dicyandiamid as a favorable source of nitrogen.

#### EXPERIMENTS WITH BUCKWHEAT.

On Oct. 2, two young plants of buckwheat, about 10 cm. long, developed in quartz sand, were put in flasks containing solutions of various strength and a great difference was observed at different concentrations:

Dicyandiamid.	Two days	Three days	Four days	Seven days	Two've days.
0.5 %	The tips of the leaves dried up.	The symptom of injury became more decisive.	dead.	—	—
0.2 %	Normal.	The tips of the leaves were little injured.	The injurious phenomena became more clear.	Almost dead.	—
0.1 %	"	"	"	"	—
0.075 %	"	Normal.	Injured a little.	Injurious phenomena advanced.	Almost dead,
0.05 %	"	"	Normal	Injured a little.	The margin of leaves became a little decolorized

8. In more diluted solution of dicyandiamid, the growth of bacteria might be luxuriant.

0.025 %	Normal.	Normal.	Normal.	Normal.	Normal.
0.01 %	"	"	"	"	"
Control (common water).	"	"	"	"	"

The next experiment was carried out in culture solution. The solution contained:

0.02 %	$\text{CaH}_4(\text{PO}_4)_2$
0.01 %	$\text{K}_2\text{SO}_4$
0.01 %	$\text{Mg SO}_4$
Trace	$\text{FeSiO}_4$

and various additions of dicyan diamid. The result was as follows:

Dicyandiamid.	Twelve days.	Sixteen days.	Twenty days.	Twenty six days.	Thirty three days.
0.05 %	The margin of the leaves was decolorized but new leaves developed.	The margin of the elder leaves became brownish.	Almost withered.	Lower leaves dropped off.	Almost dead.
0.025 %	New leaves appeared.	One plant was normal, another withered a little.	Both plants withered.	Although withered, new leaflets appeared.	as before
0.01 %	New leaves appeared.	One yielded two new leaves and other three	One bud appeared on one plant and two on other.	One bud flowered.	All buds opened.
Control.	New leaves appeared.	Both plants yielded two new leaves.	Normal.	Each plant produced a bud.	One bud opened.

A photograph was taken on Nov. 28, which is shown by the accompanying plate.

From these results, it is clear that dicyan diamid is only injurious at higher concentration for plants, properly diluted, however, it can serve as a nitrogenous nutrient.

An experiment in soil culture was also made. Each pot contained 2 kilo soil of dry land and the following general manure was applied:

Double superphosphate	...	...	...	1 g.
Potassium sulphate	...	...	...	2 „
Nitrogenous manure per pot. (N in an equivalent quantity.)				
Ammonium sulphate	...	...	...	1 g.
Sodium nitrate	...	...	...	1.29 „
Lime-nitrogen	...	...	...	1.70 „
Dicyandiamid	...	...	...	0.36 „

In this case, dicyandiamid produced a very injurious action, preventing further development of the plants in a short time. Indeed the growth remained far behind that with lime-nitrogen.

### EXPERIMENTS WITH OATS.

Similar experiments<sup>9</sup> with young plants of oats of about 10 cm. long, yielded the following results;

Dicyan- diamid	Two days.	Four days.	Six days.	Ten days.	Seventeen days.	Twenty eight days.
0.5 %	The tips of the leaves dried a little.	The leaves dried up.	dead.	—	—	—
0.2 %	withered a little.	The leaves dried up partly.	The leaves dried up.	Almost dead.	dead.	—
0.1 %	Normal.	Normal.	The tips of the leaves curled.	The tips of leaves dried up.	About the half of each leaf dried up.	The leaves are hardly green.
0.075 %	„	„	The tips of the leaves curled a little.	The tips of the leaves curled.	The tips of the leaves became brownish.	As before.
0.05 %	„	„	Normal.	The tips of the leaves curled a little	The tips became brownish.	A new leaf appeared.
0.025 %	„	„	„	Normal.	The tips decolorized slightly.	Better than the control.
0.01 %	„	„	„	„	Normal.	Better than the control.
Control.	„	„	„	„	„	Normal.

9. In these experiments, no nutrient was added.

In the next experiment, two young plants of oats, about 10 cm. long, were inserted in the flasks containing the following solutions:

	Two days	Four days.	Six days.	Ten days.	Seventeen days.
Ammonium sulphate. 0.1 %	Normal.	Normal.	Normal.	Normal.	Normal.
Sodium nitrate, 0.1 %	"	"	"	"	Best development.
Dicyandiamid. 0.1 %	The tips of the leaves withered a little.	The tips withered.	The leaves dried up partly.	About half parts of leaves dried up.	Almost dead.
Dicyandiamid. 0.05 %	Normal.	Normal.	The tips curled	The tips curled.	The tips decolorized a little. The development was a little inferior to that in ammonium sulphate.

These results show that dicyandiamid is not injurious for oats in the concentration less than 0.025%.

#### EXPERIMENTS WITH PADDY-RICE IN POTS.

Each pot contained 8 k. paddy soil<sup>10</sup>; on July 22, the young plants of rice were transplanted, three bundles in each pot, each bundle consisting of three plants. Before transplanting, the following manures were applied<sup>11</sup>. The lime-nitrogen was applied three weeks before planting, also the dicyandiamid.

	Double Superphosphat: <sup>12</sup>	Potassium sulphate	Lime-nitrogen <sup>13</sup>
A.	5 g.	10 g.	8.517 g.
B.	"	"	Ammonium sulphate 5 g.
C.	"	"	Sodium nitrate 6.436 g.
D.	"	"	Dicyandiamid <sup>14</sup> 1.774 g.

10. The soil was humus loam taken from a paddy field which had not been cultivated for several years.

11. Each pot received the same amount of N. (1.062%, per pot.)

12. This double superphosphate contained 43.9%  $P_2O_5$ .

13. This lime-nitrogen contained 12.47% N.

14. This dicyandiamid was prepared from lime-nitrogen and contained 59.88% N.

On Nov. 5, the plants were cut and weighed in the air dry state with the following result:

		Total g.	Grains g.	Straw g.
A ... ..	I.	100.00	37.2	62.8
	II.	99.5	40.0	59.5
B ... ..	I.	106.5	55.0	51.5
	II.	100.7	51.7	49.0
C ... ..	I.	45.7 <sup>15</sup>	20.5	25.2
	II.	59.5	26.0	33.5
D ... ..	I.	99.0	55.0	44.0
	II.	98.7	55.0	43.7

This result shows that dicyandiamid had produced practically the same harvest in grains, as ammonium sulphate.

In the following experiments the influence of the time of manuring on the paddy soil were tested.

#### EXPERIMENT IN POTCULTURE.

For this experiment, eighteen pots,<sup>16</sup> each containing 8 k. soil from a paddy field which had specially exhausted by raising crops for several years without using manures, were used. General manures per pot applied on June 19 were as follows:

Double superphosphate	1.265 g.
Potassium sulphate	1.029 „

Nitrogenous manures served for this experiment were as follows<sup>17</sup>

Ammonium sulphate	2.620 g.
Lime-nitrogen	4.704 „
Dicyandiamid	0.928 „

	Time of Manuring.	Time of Planting.
A.	July 10	July 10
B.	„ 3	„
C.	June 26	„
D.	„ 19	„
E.	July 10	„ [ammonium sulphate.]

15. We have repeatedly observed and mentioned that nitrates form a very poor source of N. for plants cultivated on swamp soil.

16. This experiment was carried out in two series.

17. The quantities of  $N$ ,  $P_2O_5$  and  $K_2O$  were equal in each pot, each being 0.555 g. per pot. The lime-nitrogen contained 11.8% and the dicyandiamid 59.88% of N respectively.

Three bundles of young rice-plants were planted in each pot, each bundle consisting of three plants. In the first stage of development, some retarding action of diacyandiamid and lime-nitrogen was observed in A, B and C. On Nov. 25, the plants were cut and weighed in an air dry state:

	Dicyandiamid.		Lime-nitrogen.	
	grains	straw.	grains	straw.
A.	30.5 g.	36.1 g.	28.3 g.	37.3 g.
B.	33.5 "	40.8 "	30.0 "	39.6 "
C.	31.7 "	42.1 "	29.5 "	41.1 "
D.	33.7 "	37.8 "	33.2 "	41.6 "
E.	29.5 "	38.0 "	29.5 "	38.0 "

The above result shows the average yield in two pots. This proves that diacyandiamid, like the lime-nitrogen produces a very favorable result when it is applied to the soil at least two weeks before planting.

#### EXPERIMENT IN FRAME CULTURE IN OPEN FIELDS.

This experiment was carried out in wooden frames of three square shaku (=0.83 square metres), the frames being at a distance of one metre from each other and projecting about ten centimetres above the level of the field. The depth of these frames was about seventy centimetres. The same soil used in the former pot experiment, served also in this case. As the general manures, potassium sulphate at the rate of 100 k. potash per ha, and common superphosphate at the rate of 100 k. phosphoric acid per ha. were applied. The quantities of nitrogenous manures per frame were as follows, each being at the rate of 100 k. nitrogen per ha.

Ammonium sulphate	39.24 g.
Lime-nitrogen <sup>18</sup>	66.80 "
Dicyandiamid <sup>19</sup>	17.80 "

18. This contained 12.47% N.

19. This contained 46.7% N.

	Time of Manuring.	Time of Planting.
A.	July 7	July 7
B.	June 30	"
C.	" 23	"
D.	" 16	"
E.	" 9	"
F.	" 2	"
G.	July 7	"
H.	—	"
K.	—	"

Sixteen bundles of young rice plants were transplanted in one frame, each bundle consisting of twelve plants. In the first stage of development, the plants in A were inferior to those in K, but on July 27, they commenced to show better growth than H. On August 10, there was almost no difference observed in B, C, D, E, F and G; only in A, the plants were still inferior to all others. Generally the plants manured with lime-nitrogen showed a better growth than those manured with dicyandiamid, perhaps on account of its lime-content. On Sept. 10, the number of branches was counted with the following average result:

	Number of branches per plant.	
	Lime-nitrogen.	Dicyandiamid.
A.	17.0	14.7
B.	16.9	15.5
C.	17.0	16.0
D.	18.4	18.0
E.	18.0	16.0
F.	17.0	15.5
G.	18.4	18.4 [ammonium sulphate.]
H.	13.5	13.5 [no Nitrogen.]
K.	11.9	11.9 [no Manure.]

On Nov. 28, the plants were cut and weighed in the air dry state:

THE HARVEST PER FRAME.

	Lime-nitrogen.			Dicyandiamid.		
	Fullgrains.	Emptygrains.	Straw.	Fullgrains.	Emptygrains.	Straw.
A.	197 g.	18 g.	399 g.	183 g.	12 g.	312 g.
B.	259 "	18 "	490 "	208 "	13 "	354 "
C.	260 "	18 "	508 "	209 "	13 "	350 "
D.	258 "	21 "	528 "	238 "	16 "	416 "
E.	280 "	17 "	491 "	244 "	14 "	394 "
F.	257 "	19 "	468 "	239 "	11 "	359 "
G.	266 "	16 "	482 "	266 "	16 "	482 "
H.	149 "	11 "	276 "	149 "	11 "	276 "
K.	75 "	5 "	149 "	75 "	5 "	149 "

This result again shows that dicyandiamid produced a poor result on paddy soil when planting followed immediately after manuring; however when the planting took place three or four weeks after manuring the result was nearly favorable as with ammonium sulphate. Hence we may conclude that dicyandiamid was after this time completely decomposed.

Another very interesting question was, whether dicyandiamid would be more effective in conjunction with acidic or with alkaline manure.

For this experiment four pots, each containing 2 k. paddy soil were used and the manures applied per pot were as follows:

Acidic Manure.	{ Dicyandiamid	1 g.	Alkaline Manure.	{ Dicyandiamid	1 g.
	{ Potassium sulphate	1 "		{ Potassium carbonate	0.783 "
	{ Double superphosphate	1 "		{ Dicalcium phosphate	0.95 "

On July 23, three young rice plants in one bundle were planted; on Nov. 5, were cut and weighed in the air dry state.

	Total.	Grains.	Straw.
Acidic ... ..	{ 12.2 g.	6.8 g.	5.4 g.
	{ 12.8 "	6.0 "	6.8 "
Alkaline ... ..	{ 16.5 g.	8.7 g.	7.8 g.
	{ 18.9 "	9.0 "	8.9 "



In the following experiment with millet, no paddy soil, but dry land soil (common field soil) served. Each pot contained 10 k. soil and manured with the following substances:

Acidic.	{ Dicyandiamid	5 g.	Alkaline	{ Dicyandiamid	5 g.
	{ Potassium sulphate	5 „		{ Potassium carbonate	3.91 „
	{ Double superphosphate	4 „		{ Dicalcium phosphate	3.8 „

The seeds were sown July 23 and five plants were grown in each pot. The plant growth was very much retarded, indeed the plants much injured. The plants were cut at the ripening stage and the harvest (airdry) was:

	Total.	Straw.	Ears.
Acidic ... ..	{ 6.2 g.	3.3 g.	2.9 g.
	{ 6.7 „	3.7 „	3.0 „
Alkaline ... ..	{ 8.0 g.	4.4 g.	3.6 g.
	{ 7.0 „	3.7 „	3.3 „

This result is exceedingly poor; but in further experiment with buckwheat, in which 5 g. dicyandiamid per 10 k. soil served, even poorer result was obtained, the young plants after reaching 15-16 cm. height stopping further growth and withering.

These results show that dicyandiamid is more effective in conjunction with alkaline manures.

### CONCLUSIONS.

In waterculture, dicyandiamid at the concentration of 0.01% proved to be a source of nitrogen for plants. In common soil, however, it acted poisonously at the rate of 5 g. dicyandiamid in 10 k. soil, but served as a favorable nitrogenous manure with the reduced quantities. In paddy soil the injury was less than in the soil of common dry land and when the precaution was taken, that planting was performed three weeks after manuring with dicyandiamid, no injury whatever was observed and the harvest reached nearly that obtained with the equivalent quantity of ammonium sulphate and of lime-nitrogen. This renders it very probable

that the bacteria in paddy soil sooner change this injurious compound further to innocuous compound (ammonium compound?) than in common field soil. Furthermore dicyandiamid acts as a nitrogenous manure more favorably when it was applied in conjunction with alkaline manure.

Lastly I express thanks for the assistants S. Sanada and T. Yoshida who helped me during this investigation.

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This plate shows the nutritive value of dicyandiamid in water-culture.



A. Ammonium Sulphate. B. Sodium Nitrate. C. Dicyandiamid. D. Lime-nitrogen.

This plate shows the manurial value of dicyandiamid in pot-culture



## Is Dipotassium Sulphate Physiologically Acid?

BY

K. Asō.

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When a compound of neutral reaction is transformed gradually into a compound of an acid reaction by the action of the roots, or undergoes a separation into acid and base, the latter alone or chiefly being absorbed, it is called a physiologically acid manure. This is of a special interest in the case of ammonium sulphate as Adolf Mayer has pointed out, and care has to be taken that sufficiently calcium carbonate is present in such a case in the soil. Since, however, in recent times potassium sulphate is very often applied as a manure and since the reaction of the soil has a great influence on the yield,<sup>1</sup> the question whether this salt is a physiologically acid manure, is of practical interest. Many plants require comparatively little sulphur since the albuminous matters contain rarely more than 1.8% sulphur and other organic sulphur compounds are comparatively rare in plants being generally restricted to Crucifere and to various kinds of Allium. Hence soils relatively poor in sulphur may still furnish rich crops under otherwise favorable conditions plants generally require much more potash than sulphur as shown by numerous analyses.

But it has not yet been decided whether in manuring with potassium sulphate, the sulphuric acid is absorbed in the same measure as potassa or whether by preferential absorption of potassa from neutral potassium sulphate, some monopotassium sulphate would be formed in the soil. It might also be possible that dipotassium sulphate is absorbed as entire molecule and that the sulphuric acid not needed in plants for protein formation would simply remain in the cell-sap in form of various sulphates.

1. Compare Bull. College of Agric. Tokio, Vol. VII. No. I. 1906.

The question was to be decided by sand culture in which potassium and sulphur were present in the form of dipotassium sulphate. A depression of the harvest was to be experienced, when potassium sulphate was a physiologically acid manure and no means for neutralisation was provided.

Six pots containing 2.5 k. purified quartz sand were manured with.

$\text{CaHPO}_4 + 2\text{aq.}$	0.6 g.	} per pot.
$\text{NH}_4\text{NO}_3$	1.0 "	
$\text{Fe}(\text{OH})_3$	1.0 "	
$\text{MgHPO}_4$	0.5 "	

Two pots  $A_1$  and  $A_2$  received each 0.6g.  $\text{K}_2\text{SO}_4$ ; two pots  $B_1$  and  $B_2$  received 0.5g.  $\text{K}_2\text{SO}_4 + 0.1\text{g. KHSO}_4$ ; two pots  $C_1$  and  $C_2$  received 0.5g.  $\text{K}_2\text{SO}_4 + 0.1\text{g. K}_2\text{CO}_3$ . Six seeds of oats were sown in each pot, April 20, and later on the number of plants were reduced to three, all of equal size. In the beginning of the development, the plants in  $A_1$  and  $A_2$  showed a better growth than others, but later on the plants in  $C_1$  and  $C_2$  were superior, possibly in consequence of  $A_1$  and  $A_2$  becoming acid. After ripening, the plants were cut and weighed in the airdry state with the following result.<sup>2</sup>

	Total g.	Straw g.	Grains g.	Roots g.
$A_1$	32.1	21.1	9.0	5.5
$A_2$	29.0	20.0	9.0	4.2
$B_1$	26.9	18.1	8.8	3.5
$B_2$	29.0	20.1	8.9	4.0
$C_1$	31.0	20.0	11.0	3.0
$C_2$	30.5	20.5	10.0	3.8

The difference of harvest are not very decisive, nevertheless it will be recognised that in case  $C_1$  and  $C_2$  the smallest mass of root has produced the greatest harvest in grains. It was evidently more favorable to use some alkaline substance along with potassium sulphate than to use some acid substance along with it (compare  $C_1$ ,  $C_2$  with  $B_1$ ,  $B_2$ ). Potassium sulphate appears therefore as a weak acid manure.

2. The roots were also extracted from the sand as carefully as possible and were united with the harvest.

In the second experiment, six pots containing 2.5 k. pure quartz sand were manured with the following compounds per pot,

$\text{CaHPO}_4 + 2\text{aq}$	0.6 g.
$\text{NH}_4\text{NO}_3$	1.0 "
$\text{MgHPO}_4$	0.5 "
$\text{K}_2\text{SO}_4$	0.6 "
$\text{Fe}_2\text{Cl}_6$	trace

Besides, two pots  $A_1$  and  $A_2$  received each 1.26g.  $\text{Na}_2\text{HPO}_4 + 12\text{aq}$ ;  $B_1$  and  $B_2$ , 0.67g.  $\text{NaH}_2\text{PO}_4 + 4\text{aq.}$ ;  $C_1$  and  $C_2$  1.35g.  $\text{Na}_3\text{PO}_4 + 12\text{aq}$ ; the quantity of  $\text{P}_2\text{O}_5$  being equal in each pot. Three young plants of rice were transplanted in each pot in a bundle on July 22, and harvested on Nov. 5 with the following yield:

	Total g.	Grains g.	Straw g.
$A_1$	36.5	12.0	20.5
$A_2$	35.0	14.5	20.5
$B_1$	29.5	9.8	19.7
$B_2$	27.5	9.0	18.5
$C_1$	32.0	12.0	20.0
$C_2^3$	22.0	6.0	16.0

This result shows a decisive depression of harvest when weak acid manure is applied from the start and that potassium sulphate has most manurial efficacy for rice when the other salts were applied together with it in the neutral state (compare  $A_1$   $A_2$  with  $B_1$   $B_2$  and  $C_1$ ).

As a whole it appears that the physiological acidity of dipotassium sulphate is very much less pronounced than that of ammonium sulphate, but it acts as a neutral or as weak physiologically acid manure.

3. The plants in  $C_2$  were accidentally damaged and the result is not reliable





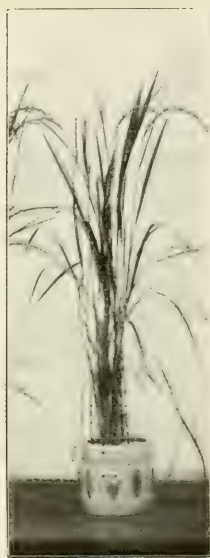
Plate XI.



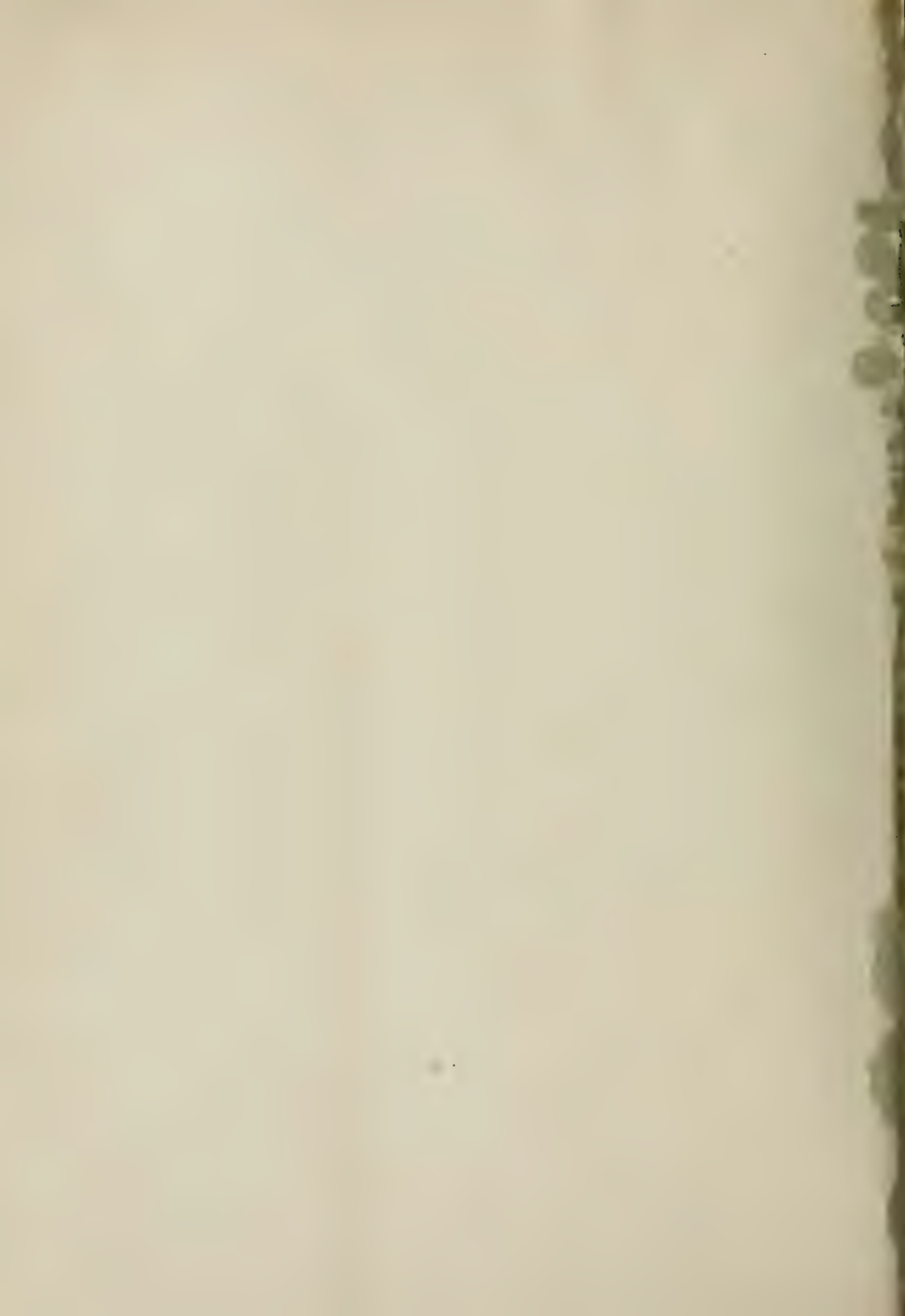
A. Neutral.



B. Acid.



C. Basic.



## Some New Varieties of *Willia Anomala* as Aging Yeast of Saké.

BY

T. Takahashi and H. Satō.

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With Plate XII.

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### Introduction.

*Willia anomala*, discovered by WILL<sup>1</sup> and formerly called *Saccharomyces anomalus*, forms now a large group of *Saccharomyces*. HANSEN<sup>2</sup> (1891) first described this organism under the name of *Sacch. anomalus*. P. LINDNER<sup>3</sup> (1892) found it on a green malt<sup>4</sup> and further he found a new variety *Willia anomala* var *BELGICA*<sup>4</sup> in Belgian beer. CHIRZASZCZ (1902)<sup>5</sup> reports that he has found *sacch. anomalus* on the surface of the barley, and JØRGENSEN<sup>6</sup> (1898) mentions a variety of *sacch. anomalus* which cause a turbidity in English beer (bottom fermentation system). From a Canadian cheese factory HARRISON<sup>7</sup> found a variety of *sacch. ano-*

1. Will: Zeit. f. d. ges. Brau. 1892. p. 75.
2. Hansen: Comp. rend. labo. Carlb. 1891.
3. Lindner: Woch. für B. 1892. No. 4. p. 75.
4. „ : Mikrosk. Betriebskont. 1898.
5. Chirzaszcz: Woch. f. Brau. 1902. p. 590.
6. Jørgensen: Mikroorganismen. 4 Auf. p. 238.
4. Lindner: Woch. f. B. 1900. Nr. 49-51.
7. Harrison: Cent. f. Bak. Bd. IX. p. 206.

which gives a "bitter aroma" to the milk. WILL<sup>8</sup> observed an anomalous type yeast in the wort of the cool ship, younger beer, beet-sugar and refined cane sugar. L. STEUBER<sup>9</sup> made studies on his three varieties of *Willia anomala* and one variety discovered by WILL from cherries.

MEISSNER<sup>10</sup> mentions three different types of *anomala* isolated from beer, wine must and the soil from their gigantic colonies on wine must gelatine. KUJAWSKI<sup>11</sup> found a variety of *anomala* in plum-sauce, but his description is short. H. ZIKES<sup>12</sup> found in his soil analysis a new variety of *Willia anomala*, which forming a *slimy covering* on several kinds of nutriments and thereby distinguishes itself from other varieties of *Willia anomala*. KLOCKER and SCHIONING<sup>13</sup> mention the presence of *Willia anomala* in the so-called "Taka-Koji". KOZAI<sup>14</sup> in his investigations on the microbes of "Koji," reports the occurrence of a variety of *Willia anomala*, causing a feeble alcoholic fermentation and producing acetic ether in beer wort. K. SARRO<sup>15</sup> isolated from Saké a kind of *Willia anomala*, which produced acetic and butyric ethers in beer wort or in Koji extract and was capable of fermenting dextrose, levulose, saccharose, galactose and sparingly also maltose. Quite recently S. FUKUMOTO<sup>16</sup> isolated five varieties of *Willia anomala* from "Moromi-mash." All of them develops well only in koji-extract containing less alcohol than 11 vol %, and one of them produces amylacetate.

8. Will: Cent. f. Bak. Abt. II, Bd. VI, p. 219.

9. Steuber: Zeit. f. das g. Brau. 1900, p. 3, 17, 33.

10. Meissner: Land. Jahr. 1901.

11. Kujawski: Zeit. f. d. g. B. Bd. XXIII, 1900, p. 111.

12. Zikes: Cent. für Bak. 1906, II Ab. Bd. XVII, No. 416-S, 97-111.

13. Klöcker u. Schioning: Cent. f. Bak. 1895, II Ab. Bd. I, S. 777-782.

14. Kozai: Cent. f. B, II Ab. Bd. VI, 1900, p. 335.

15. Saitō: Journal of Imp. Univ. Tokyo, College of Science. 1904, Vol. XIX.

16. Fukumoto: Jōzōkyokaizatsushi. 1910, Vol. I.

## PART I.

It is a well known fact that a new or freshly prepared saké has a very sharp taste and is too strong as a beverage for refined people, who consumes almost exclusively thoroughly ripened saké. This process of *after ripening* or *aging* occurs during the storage, which takes place after clarifying and continues from spring to autumn. During this storage which continues all through the hottest part of the year, the temperature of the saké in the vat is relatively high <sup>17</sup> and the chemical changes in the composition of the new saké must proceed at a rather quick pace and complete the ripening. But the exact nature of these changes has not been known. The authors isolated four varieties of *Willia anomala* from a deposit <sup>18</sup> formed on the bottom of the vat during the storage. They are described below. Only *The var. III and IV form amylicetate*.

No. 1. *Willia anomala* Var. I.

I. Form and Size: (a) *Young culture*:—Koji-extract, 7 days culture at 23-25° C. The cells are round ( $3 \times 3 \mu$  or  $5 \times 5 \mu$ ), elliptical ( $5 \times 6.5$ - $7.5 \mu$  or  $5 \times 8 \mu$ ), club shaped ( $12.5 \times 5$ - $6 \mu$ ), or oval ( $7.5 \times 5 \mu$ ) and many of them contain one or two fat globules. The cells from surface cultures on Koji-extract agar (27 days at room temp.) are small and round ( $2.5 \times 2.5 \mu$ ), small and elliptical ( $3 \times 2.5 \mu$ ), or somewhat elongated mostly, but some of them are large and round ( $5 \times 5 \mu$ ), oval ( $3 \times 6.5 \mu$ ) or elliptical ( $5 \times 4.5 \mu$ ), and contain glycogen. Old culture: The cells from surface growths on saké-agar (10 months) are very small and round ( $2.5 \times 2.5 \mu$ ) or small and elliptical mostly and large cells of  $4 \times 5$ ,  $4 \times 4$ ,

17. In summer time the temp. of saké in the storage vat is 24-28°C.

18. The deposit of a saké from a factory of "Nishinomiya," "Nada."

or  $10 \times 2.5 \mu$  are very rare. Some of these cells contain 1-4 hat shaped spores with  $2.5 \mu$  ledge length and  $1.5 \mu$  high.

II. Growth: In Saké-agar plate culture (10 days at  $25^{\circ}$  C) there was found a white flat colony<sup>19</sup> with some folds in the centre and feathery growth at the margin. *Surface culture*: A white waxy covering with a somewhat streamy appearance margin with the presence of a promycelium, was formed on saké-agar, (52 days from June to July at room temp.). A greyish white waxy and almost flat growth with promycellium on the thinner part of the medium was formed (3 months during summer). A smooth pasty covering was found on Moromi-agar. (10 days at  $20^{\circ}$  C). The covering on the thick part of the medium stretches out considerably with a wavy margin, while on the thinner part it forms a white, chalky crust. A white, chalky, thick, folded film was observed on the condensed water, which contained the deposited cells with a sign of fermentation. After 2 months more the covering lost its lustre and became coarse. A white waxy covering with concavity along the streaks, and forming folded streams at the margin, was observed on saké-gelatine culture, (one month at room temp.). A white chalky growth was also formed on the thinner part of the medium. *Fluid culture*: In beer wort (3 days at  $23^{\circ}$  C) it forms a thin film with somewhat massive deposit and on shaping an energetic disengagement of gas was observed. In koji-extract ( $10^{-3}$  B, 16 days at  $15-16^{\circ}$  C) it forms a very thin film forming a foam and a deposit under clear fluid, with the production of acetic and other esters. Some of the cells in the film of koji-extract culture (80 days) stain red<sup>20a</sup> with methyleneblue.

III. Ester formation and its relation to carbon-source. As culture

19. Some of the colonies grow in three directions, as is usual in the common yeast.

19a. This phenomenon will be dealt with further on. *Soy-yeast behaves similarly toward methyleneblue.*

media solutions were used containing as carbon-source substances other than carbohydrates.

TABLE I.

Solutions.	Condition.	Remarks.
No. I. <sup>20</sup> ... ..	In test tube. 2 months at room temp.: July to August.	Forms white film and a ring with massive deposit, and produces fruit esters.
No. II. <sup>21</sup> ... ..	Do.	Forms white film and ring with considerable deposit. The ring does not break even on shaking.
No. II. ... ..	10 days at 25-27°C.	Forms no film but with a trace of acetic ester flavor.
No. III. <sup>22</sup> ... ..	In test tube. 2 months at room temp.	Forms thin white film and ring with noticeable deposit.
No. II. ... ..	In sealed flask. 3 months at 23-25°C.	No film, but an energetic production of acetic ester.

Thus, this variety forms *esters from acetate and alcohol derived from the amino-acids* formed by autolyses. Further, it is also probable that *ester is formed from the acetate and alcohol originally given in the nutrient*. The function of ammonia as a nitrogen-source is also proved by these cultures.

A further experiment was made in the same direction with different culture media.

20. Solution No. I. contained:  $\text{KH}_2\text{PO}_4$ , 0.04g,  $\text{K}_2\text{SO}_4$ , 0.02g,  $\text{MgSO}_4$ , 0.0003g,  $\text{C}_2\text{H}_5\text{O}_2$ , ( $\text{NH}_4\text{OH}$ ), 2g. Water 100c.c.

21. Solution No. II. contained: 5% of ethyl-alcohol in 100c.c. of No. I. solution.

22. Solution No. III. contained: 0.05% of amyl-alcohol in 100c.c. of solution No. I.

TABLE II.

Solution.	Condition.	Remarks.
No. IV. <sup>23</sup> ... ..	14 days at 17-18°C.	Forms a thin film with a heavy deposit and vigorous production of acetic and other esters.
No. V. <sup>24</sup> ... ..	Do.	A white thin film was formed ascending to the wall, with deposit and ester production.
No. V. ... ..	In sealed flask during 2 months at 23-25°C.	Forms thin film accompanying the formation of fruits esters.
No. VI. <sup>25</sup> ... ..	Do.	Forms thin film with deposit and trace of fruit ester. The film is thinner than in No. 4, which did not contain amyl-alcohol.
No. VII. <sup>26</sup> ... ..	Do.	Forms relatively thick (thickest among four varieties) white film ascending the wall of the flask, with heavy deposit and a vigorous production of acetic and fruit esters.
No. VIII. <sup>27</sup> ... ..	10 days at 25-26°C.	Forms fine folded film ascending the tube wall, but without flavour.
No. IX. <sup>28</sup> ... ..	4 days at 24.5°C.	Forms fine folded film, ascending the wall, with trace of deposit. Deposit increased after 9 days.

Thus, it is plain that the yeast assimilate carbon from butyrate, succinate or free succinic acid and lactate, and that the formation of ester flavour always takes place except in the case of lactate. So that, it is highly probable that in the presence of the latter the yeast has not the

23. Solution IV. contained  $\text{KH}_2\text{PO}_4$ , 0.04g,  $\text{CaSO}_4$ , 0.02g  $\text{MgSO}_4$ , 0.0003g.  $\text{NH}_4\text{C}_2\text{H}_3\text{CO}_2$ , 0.5 c.c. and alcohol (absolute) 8c.c. in 100c.c. of water.

26. Solution VII. was prepared by adding 0.05 vol% amyl-alcohol to solution No. V.

24. Solution No. V. contained 0.5% of ammonium succinate instead of the ammonium butyrate of solution No. IV.

25. Solution No. VI was prepared by adding 0.05 vol% of amyl-alcohol to solution No. IV.

27. Solution VIII. contained 0.5c.c. of ammonium lactate instead of the ammonium butyrate solution No. IV.

28. It contained  $\text{KH}_2\text{PO}_4$ , 0.04g, Asparagin 2.5g,  $\text{K}_2\text{SO}_4$ , 0.02g,  $\text{MgSO}_4$ , 0.0003g, succinic acid 0.05g, ethyl-alcohol 5c.c. and water. (to make 100c.c.)



power of forming an alcohol of an agreeable odour by combining with lactic acid, or of forming any acid which gives fragrant substance in combining with ethyl alcohol.

Further, a remarkable fact is that the production of aroma was greater in the culture media containing both amyl-alcohol and succinate than in those containing only the latter (solution No. V). Moreover, it is very interesting to note that fruit esters was formed in the solution No. I, in which the only carbon source was ammonium acetate. So that it is highly probable that alcohol, which favours the formation of esters, must be derived from the amino acid formed by autolyses.

IV. Spore formation. The hat shaped spores are formed on the gypsum blocks after 38 hours at 27° C. One to four spores are found in a cell.

V. Fermentation products. In the distillate of a 6 days old koji-extract culture (10° B) at 22-20° C, there was found fusel oil, methyl, alcohol, acetone and methyl-lactate.

The culture contained 2.72% ethyl alcohol, 0.209% total acid (as succinic acid)<sup>28</sup> and 0.638% total esters (as ethyl-succinate)<sup>29</sup>.

VI. Behavior towards carbohydrates<sup>30</sup>:—Cultures at 23.5-24° C.

28. 29. In the case of other varieties described below total acid and total esters are calculated as succinic acid and ethyl-succinate respectively.

30. Such carbohydrates as arabinose, xylose, glucose, levulose, galactose, raffinose, lactose maltose and  $\alpha$ -methylglucoside and also glycerin were dissolved in the proportion of 2% in Hayducks solution devoid of sugar, while in the case of cane sugar 5% was the strength used

Substances.	Remarks.
Arabinose ... ..	Forms half film after 3 days, completed after 7 days; but it is very thin and not folded, with a trace of deposit.
Xylose... ..	Very thin film begins to form after 3 days and is completed after 7 days with a trace of deposit.
Fructose ... ..	Forms a very thin and almost transparent and non-folded film with peposit and turbid fluid after 3 days. A feeble fermentation was shown by the evolution of bubbles which increased on shaking. Produces fruit esters which remained even after 10 days.
Glucose ... ..	Forms very thin film and rings; fluid turbid with dense foams and white deposit after 4 days. When shaken gas bubbles were vigorously evolved.
Galactose ... ..	Forms very thin and almost transparent film, but giving no deposit after 3 days. Davy's test for alcohol gave a positive result after 14 days culture.
Saccharose ... ..	Forms thin white film and foam with turbid fluid and a deposit (3 days culture), which does not scatter when shaken.
Lactose ... ..	Forms island-like film with no deposit and turbidity after 3 days. A trace of deposit was formed after 14 days but Davy's test for alcohol gave a negative result. The degree of the growth was almost equal to xylose-solution.
Maltose ... ..	Forms white non-folded film ascending the wall after 3 days. The deposit increased after 14 days. Fruit ester was present after 10 days but Davy's test for alcohol gave no result.
Raffinose ... ..	Forms thin white non-folded film with white deposit after 4 days. After 10 days gave faint reaction for alcohol.
Glycerin ... ..	Forms very thin film with trace of deposit which does not change when shaken.
$\alpha$ -Methylglucoside ... ..	Forms very thin film and a trace of deposit after 3 days.

Thus, this variety assimilates xylose, arabinose, fructose, glucose, galactose, saccharose, lactose, maltose, raffinose glycerin and  $\alpha$ -methyl glucoside; especially in solutions containing fructose, glucose, saccharose the growth is vigorous and the ferment good. But xylose and lactose are less favourable for its growth and galactose is fermented sparingly.

VII. Assimilation of nitrogen compounds and difference between glycerin and cane-sugar as regards ester formation. Two series of the solutions were prepared one containing glycerin and the others containing saccharose, with four kinds of N-source in 4 solutions in each series.

Substances. <sup>31</sup>	Remarks.
Asparagin +glycerin ...	Forms white foldedless film ascending the wall (10 days at 23-24°C). There was deposit but not ester flavor; while in the culture of Hayduck's solution, a vigorous formation of fruit ester took place.
Peptone <sup>31b</sup> +glycerin ...	Forms very thin film with ring and heavy deposit but no ester flavor, (10 days at 23-24°C), while in peptone Hayduck's solution there was an intense ester flavor.
Ammonium-phosphate <sup>31c</sup> +glycerin	Forms thin film with ring shaped growth and deposit (10 days at 23-24°C). The film formed on ammonium phosphate Hayduck's solution was thinner than that of this solution, but fruit ester formed only in the former.
Potassiumnitrate <sup>31d</sup> ...	Forms thin film ascending the wall but no deposit after 3 days at 22-23°C. After 2 month there was formed a heavy deposit but there was no flavor of ester, while in the culture of KNO <sub>3</sub> -Hayduck's solution, ester flavor was conspicuous.

Thus, this variety assimilated asparagin, peptone, ammonium and nitrate nitrogen. *Ester formation was strongly dependent upon the presence of cane sugar<sup>32</sup> but no on glycerin.* This fact explains well the fact that this yeast when taken glycerin as a carbon source has not the power of forming acid, which favours the formation of ester in combining alcohol, derivable from amino-acid by autolysis.

31. Nitrogen compound was dissolved in Hayduck's mineral solution containing 2% of glycerin instead of saccharose:— peptone 1%, asparagin 2.5%, ammonium phosphate 1%, K-nitrate 1%.

31. b. Peptone Hayduck's solution is prepared by dissolving peptone instead of asparagin in ordinary Hayduck's solution. So contains saccharose instead of glycerin of above solution.

31. c. Ammonium Phosphate Hayduck's solution contains ammonium phosphate instead of asparagin of ordinary Hayduck's solution.

31. d. Potassium nitrate Hayduck's solution is prepared by dissolving KNO<sub>3</sub> instead of the asparagin of ordinary Hayduck's solution.

VIII. Proteolysis. The experiment was carried on in two different ways,<sup>33</sup> by stab culture on the one hand and by uniformly distributing the yeast in the medium, using neutral koji-extract gelatine (10% gelatine). The temperature of the room was 17-20° C.

Stab culture.	Uniformly distributed.
After 40 days. No liquefaction ... ..	No liquefaction.
After 56 days. Do. ... ..	About 2 c.c. of gelatin in test tube dissolved.
After 70 days. Do. ... ..	Dissolved part increased.

IX. Assimilation of amino-acids. In koji-extract culture (10° B) after 55 days (one month at 20° C and 25 days at 10-16° C) amino-acids were determined according to SORESENSEN'S<sup>34</sup> method with the following results.

Amino-acids in original solution ..... 0.123% as glycocoll.

Amino-acids after culture ..... 0.0052% as glycocoll.

Amino-acids assimilated ..... 0.116% as glycocoll.

Therefore, almost all the amino-acids contained in the original solution must have been assimilated.

X. Optimum temperature for growth: The optimum temperature<sup>34</sup> lies about 30-31° C.

XI. Death temperature: In diluted saké (saké to water 1:1) or beer wort heating to 56-57° C for 30 minutes kills the yeast, but not in

32. Other fermentable carbohydrates also contribute to the ester formation.

33. Will. Studien über Proteolyse durch Hefen. Cent für Bact. u. Paras. 2. Abt. Bd. VII. S. 794-809.

34. Bericht d. Deut. chem. Ges. 1909.

34. To ascertain this temperatures of 20-22°C., 24-25°C., 28-32.5°C., 30-31°C., 50°C. were tried for all the varieties described below.

koji-extract or yeast-water. Heating to 57-58° C for 5 minutes death will ensure in diluted saké but in koji-extracts it is retarded.

## XII. Limit of alcohol contents for growth.

In koji-extract containing 10% of alcohol, it forms a foamy film with deposit after 6 days at 27° C; while in koji-extract containing 15% of alcohol there was no film or deposit under the same condition. The latter culture was then kept at the room temperature (17-20° C), and after 31 days there was formed a trace of deposit which increased very slowly.

Thus, this variety decidedly belongs to the group of *Willia anomala*, forming acetic—and fruit esters in cultures made in solutions containing sugar or ethylalcohol and salt of organic acid or simply organic acid salt. And above all, the ester formation is best in solutions containing ammonium butyrate. Moreover, the assimilation of amino acid is more conspicuous than in the case of sacch. sake, wine yeast or beer yeast<sup>34</sup>.

## No. 2. *Willia anomala* Var. II.

1. Form and size: (a) *Young culture*:—Koji-extract culture (7 days at 23-25° C). In the film smaller cells predominate which are either round ( $2 \times 2 \mu$ ), ellipsoidal ( $2.5 \times 3 \mu$ ) (or  $4 \times 5 \mu$ ), but somewhat larger pear shaped ( $7.5 \times 5$  or  $7.5 \times 2.5 \mu$ ), filamental ( $7.5 \times 2.5 \mu$ ), or wedge-shaped ( $10 \times 3.5$  or  $10 \times 5 \mu$ ) ones also occur frequently. Many of the cells contain 1-2 fat globules. (b) *Old culture*:—Surface culture on saké-agar after 10 months. The smaller cells predominate ( $4 \times 3 \mu$ ), but large round cells ( $8 \times 8 \mu$ ), filamental cells ( $5 \times 2 \mu$  or  $10 \times 5 \mu$ ) or club shaped cells ( $10 \times 2.5$ ) occur frequently. The spore holding cells occur very rarely and the ledge of the hat shaped spores is sometimes not evident.

Surface culture on koji-extract agar (27 days at room temperature):—Smaller round, elliptical, oval shapes predominate but large and long

34. b. See this Journal p. 279 (Takahashi and Yamamoto's paper).

elliptical, oval, sausage or club shaped cells occur rarely. The glycogen reaction is evident in every large cell. The sizes of the cells are:  $2.5 \times 2.5 \mu$ ,  $5 \times 3 \mu$ ,  $10 \times 3 \mu$ ,  $12.5 \times 4 \mu$ ,  $12.5 \times 2.5 \mu$ .

II. Growth: A faintly dirty white flat colony, with folded centre and streamy growth on the margin, appears on saké agar plate culture (13 days at  $25^{\circ} \text{C}$ ). *Surface culture*: A greyish white waxy covering was found on saké-agar. The covering changed to a chalky white fine striated growth, with conspicuous promycellium. The covering on koji-extract-agar<sup>35</sup> is almost same as that of variety I, but the difference consists in that this variety does not form a promycellium. Moreover, a more or less dirty coloration of the covering in this medium distinguishes it from the other three. The covering forced on Moromi-agar (10 days at  $20^{\circ} \text{C}$ ) was very like that of variety I, with the only difference that the growth on the thinner part of the medium develops chalky white foulds.

The growth on saké-gelatine (one month at room temp.) was almost same as that of variety I.

*Fluid culture*: In beer wort (3 days at  $23^{\circ} \text{C}$ ) it forms a thin film with moderate deposit. A vigorous evolution of  $\text{CO}_2$  gas was observable when shaken. In koji-extract ( $10^{\circ} \text{B}$ , 16 days at  $15\text{--}16^{\circ} \text{C}$ ) it forms a similar growth, but there was no foam in this variety. A faintly yellow film is formed with vigorous fermentation on koji-extract kept at  $23\text{--}25^{\circ} \text{C}$  for 3 days, or at  $17^{\circ} \text{C}$  for 7 days. A few of the film cells stain red with methylenblue. (80 days culture in koji-extract.)

III. Ester formation and its relation to carbon-source. The solutions used were the same as those mentioned connection with the foregoing experiments.

35. The duration and temperature of the cultivation are exactly the same as in the case of the variety I.

Solutions.	Conditions.	Remarks.
No. I. ... ..	In test tube. 2 months at room temp: July to August.	Same as variety I.
No. II. ... ..	Do.	Forms very thin white film and fruit ester but with no deposit.
No. II. ... ..	10 days at 25-27°C.	Almost same as variety I, but with formation of free acetic flavour.
No. II. ... ..	In sealed flask 3 months at 23-25°C.	Forms no film but vigorous acetic ester flavour.
No. III. ... ..	In test tube. 2 months at room temp: July to August.	Forms thin film and ring with deposit.
No. IV. ... ..	14 days at 17°C.	Forms no film (difference from var. I), but heavy deposit and flavor of amyl ester.
No. V. ... ..	Do.	Forms very thin (the thinnest of the four varieties) film ascending the wall. Forms deposit.
No. V. ... ..	In sealed flask: for 2 months at 23-25°C.	Same as variety I.
No. VI. ... ..	Do.	Forms thin film and deposit with amyl ester.
No. VII. ... ..	Do.	Forms very thin film as in the case of solution No. V, the thinnest of the four varieties.
No. VIII. ... ..	10 days at 25-26°C.	Same as variety I.
No. IX. ... ..	4 days at 24.5°C.	Forms non-folded film with less ascending habit as compared with variety I.

Thus, this variety assimilates butyrate, succinate or free succinic acid and lactate as carbon source, and forms fragrant esters from alcohol and above mentioned salts with the exception of the lactate (same as variety I). The formation of amylester was most conspicuous when amylalcohol was added to solution No. IV. (butyrate solution). Acetic ester was formed in acetate solution (sol. No. I) devoid of ethylalcohol (same as variety I). The production of ester flavour was greatest in butyrate solution (No. VI. solutions).



IV. Spore formation. The spores are formed on the gypsum block after 38 hours at 27° C. One or two spores are found in a cell.

V. Fermentation products. In the distillate of koji-extract culture<sup>36</sup>, there was found fusel oil, acetic ester, methylalcohol, acetone (?) and ethylalcohol (3.42 vol. %). The quantities of acid and esters were:

0.315% Total ester (as ethyl-succinate).

0.0177% Total acid (as succinic acid).

VI. Behavior towards carbohydrates:

Substances.	Remarks.
Arabinose ... ..	Same as in variety I. (7 days at 23.5-24°C).
Xylose ... ..	Forms very thin film and a trace of ring, but without deposit. (3 days at 23.0-24°C). Same even after 7 days.
Fructose ... ..	The growth was almost same as in variety I, but the film was some-what thicker.
Glucose ... ..	Same as in variety I, with the difference that the fluid remained clear and the deposit was small. (4 days at 23.5-24°C).
Galactose ... ..	Forms film with faint folds with ring and trace of deposit. (4 days at 23.5-24.5°C).
Saccharose ... ..	Almost same as in variety I, except that the ring was thicker. (4 days at 23.5-24°C).
Lactose ... ..	Same as in variety I. (4 days at 23.5-24°C). Davy's test (or alcohol) gave a positive result after 10 days.
Maltose ... ..	Same as in variety I. (3 days at 23.5-25°C). Davy's test for alcohol gave a positive result after 10 days.
Raffinose ... ..	Almost same as in variety I. (3 days at 23.5-25°C). except that the ring was thicker. Davy's test gave a negative result after 10 days.
Glycerin ... ..	Forms a thicker ring than in variety I, and the deposit scatters in the fluid when shaken. (3 days at 23.5-25°C).
$\alpha$ -methylglucoside ... ..	Almost same as in variety I, but the film is thicker.

36. The conditions of culture were the same as for variety I.



Thus, this variety is almost exactly like var. I so far as assimilation of carbohydrates is concerned, but the film on xylose solution, both the film and ring on galactose, cane sugar, raffinose, glycerin,  $\alpha$ -methylglucoside solution are thicker and more prominent than in variety I. Another distinguishing point is that *raffinose is fermented by variety I but now by this*, and that maltose is fermented by this variety but not by variety I.

VII. Assimilation of nitrogen compounds and difference between glycerin and cane sugar as regards ester formation.

Substances.	Remarks.
Asparagin + glycerin ...	Forms almost the same film, but some what thicker than in variety I. No ester flavor was perceivable. (10 days at 23-24°C). while in ordinary Hayduck's solution it was copious.
Peptone + glycerin ...	Makes almost the same growth as variety I, but the ring is thicker No ester flavour was developed. (10 days at 23-24°C). But in the culture of peptone Hayduck's solution, there was a development of ester flavour.
Ammoniumphosphate + glycerin	Forms same film as variety I. except that the ring is thicker (10 days at 23-24°C). A vigorous development of the ester flavour was perceivable in the culture of the ammonium-phosphate Hayduck's solution, while growth was conspicuous in this solution. <sup>37</sup> (containing glycerin instead of cane sugar).
K-nitrate + glycerin...	Forms half covered film. (3 days at 22-23°C). After 2 months a trace of butyric acid <sup>38</sup> flavour was perceivable, with considerable deposit, while in the culture of K-nitrate Hayduck's solution ester flavour was perceivable.

Thus, variety II assimilates asparagin, peptone, ammonium and nitrate nitrogen. The formation of ester depends upon cane sugar, but not on glycerin, as stated above.

37. In this property it agrees with variety I.

38. The production of butyric acid was observed by meissner's mycoderma. (sporeless film yeast).

## VIII. PROTEOLYSIS.

Stab culture.					Uniformly distributed
After 40 days.	No liquefaction	...	...	...	No liquefaction.
After 56 days.	Do.	...	...	...	About 2 c.c. of gelatine in test tube dissolved.
After 70 days.	Do.	...	...	...	Dissolvee part increased.

## IX. Assimilation of amino-acids.

Amino-acids in original koji-extract.... 0.123% (as glycocoll)

Amino-acids after fermentation..... 0.026% (as glycocoll)

Amino-acids assimilated ..... 0.097% (as glycocoll)

Thus the larger part of the amino acid contained in the original koji-extract was assimilated.

X. Optimum temperature for growth. It lies between 30-31° C.

XI. Death temperature: In diluted saké (50% water) heating to 56-57° C for 30 minutes kills the cells, but not in beer wort, Koji-extract or yeast-water. Death will ensue in diluted sake after heating to 57-58° C for five minutes, but not in Koji-extract.

XII. Limit of alcohol content for growth.

In Koji-extract containing 10% of alcohol, it makes almost the same growth after 6 days at 27° C, with vigorous fermentation; but when the alcohol was increased to 15% there was no film or deposit under the same conditions. After this observation the culture was held at the room temperature of 17-20° C, and after 36 days there was formed a film (the thickest of the 4 varieties) and the turbidity was increased.

The above facts show that this yeast is also a variety of *Willia anomala*, and its main distinguishing points as compared with variety I are the form of the colonies, bad growth in solutions No. II, No. IV,

or No. VI, inability of fermenting raffinose, and maltose fermenting property and its greater resistance to heat and alcohol.

### No. 3. *Willia anomala* Var. III.

I. Form and Size: (a) *Young culture*:—Koji-extract culture. (7 days at 23-25° C). In the film the predominant cells are filamental ( $10 \times 2.5 \mu$ ,  $7.5 \times 2.5 \mu$ ,  $12.5 \times 2.5 \mu$ ), and round ( $3 \times 3 \mu$ ) or elliptical ( $4 \times 3.5 \mu$ ) cells occur very seldom. Cells, containing fat globules are not found. (b). *Old culture*. In the surface culture on saké-agar after 4 months, the majority of the cells are elliptical ( $3 \times 4 \mu$ ), but long ones ( $12.5 \times 2.5 \mu$ ,  $6 \times 3 \mu$ ,  $5 \times 3 \mu$ ,  $13 \times 3 \mu$ ) also occur rarely. Spores have not been found even in such old cultures.

In the surface culture of Koji-extract-agar (27 days at room temperature) the predominant cells were small elliptical ( $2 \times 3 \mu$ ), or long elliptical ones and large round ( $5 \times 5 \mu$ ) or club-shaped ( $15 \times 2.5$ ,  $15 \times 4 \mu$ ) cells occur very rarely. The majority of the cells gave glycogen reaction.

II. Growth. The colony in sake-agar *plate culture* (13 days at 25° C) was dirty white (same as variety II). The central part of the colony was elevated somewhat and there were no folds but the appearance was granular. Both acetic ester and acetic amylester flavours were perceived. *Surface culture*. A greyish white covering with more or less mesentery like folds was found on saké-agar (52 days at room temp.). The marginal part of the covering was composed of streamy growth but there was no formation of the promycelium. On the surface of the condensed water there was formed a white folded film. On Koji-extract agar (3 months at room temp. July to September), there was formed a greyish white waxy and almost smooth covering. A promycelial growth was observed in the thinner part of the growth. The covering formed on Moromi agar (10 days at 20° C) was chalky white with fine folds. On

the surface of the condensed water, there was formed a white film with deposit and fermentation was accompanied by turbidity. On sake-gelatin (one month at 11-15° C) it forms a dry chalky (on the thin part of the medium) or somewhat waxy (on thick part of the medium) covering. The central part is elevated and surrounded by many layers of growth and fine radiating lines of sprouting were observable at the margin. *Fluid culture*: In beer wort (3 days at 23° C) it forms a thicker film than the foregoing two varieties. A vigorous evolution of CO<sub>2</sub>-gas was observed when shaken. In Koji-extract (10° B, 16 days at 15-16° C) it forms a white and finely folded film which ascends the wall, with deposit and acetic—and other ester flavour. A few of the film cells *stain red* with methylenblue (80 days culture in Koji-extract).

### III. Ester formation and its relation to carbon-source.

Solutions.	Conditions.	Remarks.
No. I ... ..	In test tube. 2 months at room temp.: July to August.	Forms no film with a trace of deposit. So quite differently from varieties I and II.
No. II... ..	Do.	Same as in variety II.
No. II ... ..	12 days at 25-27.5°C.	Forms deposit but no film. Acetic ester flavour was pronounced, which distinguishes this yeast from var. II.
No. II... ..	In sealed flask. 3 months at 23-25°C.	Forms no film but a trace of acetic ester flavour.
No. III ... ..	In test tube 2 months at room temp.	Forms a white film and ring with deposit (difference from var. II) and trace of fruit ester.
No. IV ... ..	14 days at 17-18°C.	Forms a trace of film with heavy deposit and amylester and by the former character distinguished it from var. I or var. II.
No. V ... ..	14 days at 17°C.	Forms a thin but spotted film (difference from var I, II and IV), and ester flavour.
No. V ... ..	In sealed flask during 2 months at 23-25°C.	Flavour of amylester is perceivable but on film is formed.

Solutions.	Conditions.	Remarks
No. VI ... ..	Do.	Forms trace of thin film with deposit, which is rather copious than in the solution devoid of amylalcohol.
No. VII ... ..	Do.	Forms a thin <sup>39</sup> film with white spots but no flavour (difference from variety I).
No. VIII ... ..	10 days at 25-26°C.	Forms almost the same film as var. I and II. Fruit ester flavour is conspicuous.
No. IX ... ..	4 days at 27-5°C.	Forms a similar film but with more conspicuous spots than in var. II. Deposit and turbidity are also more conspicuous than in var. II.

Thus the variety III does not form a film in solution No. I, which does not contain ethylalcohol; and this property distinguishes from var. I and II. In other words, this variety grows very little in the solutions containing ammonium acetate as the only carbon-source; while if a small quantity of ethylalcohol is added the growth is good, indicating that ethylalcohol may be a carbon source to this variety. Moreover the non-development of ethylalcohol is added the growth is good, *indicating that ethylalcohol may be a carbon source to this variety.* Moreover the non-development this variety as carbon-source was also observed. As regards butyrate and succinate this variety stands between var. I and var. II. The *formation of ester from lactate* containing solution (No. VIII) by this variety distinguishes it from var. I and var. II.

IV. Spore formation. It forms on gypsum block after 38 hours at 27° C. Four spores are contained in each cell.

V. Fermentation products. In the distillate of Koji-extract culture, there was found fusel oil, methylalcohol and acetic ester but not acetone. Beside 2.02% ethyl alcohol, 0.209% total acid and 0.259% of ester (as ethylsuccinate).

39). Thicker than in var. II, thinner than in var. I.

## VI. Behavior towards carbohydrates.

Substances.	Remarks.
Arabinose ... ..	Forms island-like growths on the surface of the solution (3 days at 23.5-24°C). The film is not complete even after 7 days i.e. growth is the poorest of the four varieties.
Xylose... ..	Same as variety I.
Fructose ... ..	Forms half covered film, but thicker than in variety II (3 days at 23.5-24°C). There was no deposit or evolution of gas, indicating that fermentation is vigorous less than in the two varieties described above. Gave a weak Davy's reaction for alcohol after 10 days.
Glucose ... ..	Film very similar to that of var. I and II. Fluid is turbid with heavy deposit and vigorous evolution of CO <sub>2</sub> gas (4 days at 23.5-24°C).
Galactose ... ..	Same as in var. I (4 days at 25.5-24°C). Davy's test for alcohol gave a positive result after 10 days;—same as in var. I and II.
Cane sugar... ..	Same as in var. II.
Lactose ... ..	Forms island-like growths on the surface of the solution as in var. I and II. Gave no reaction for alcohol with Davy's test.
Maltose ... ..	Makes almost the same growth as var. I and II, but the film is thicker (3 days at 32.5-24°C). Davy's reaction for alcohol after 10 days was faint as in the foregoing varieties.
Raffinose ... ..	Forms almost the same film as variety I (4 days at 23.5-24°C), but it was not complete. Davy's test gave a negative result after 10 days.
Glycerin ... ..	Same as in variety II (3 days at 23.5-24°C).
<i>α</i> -methylglucoside ... ..	The film formed was almost same as in var. II, but it was thicker than in variety I (3 days at 23.5-24°C).

Thus, the assimilation of xylose and lactose by this variety was feeble as in the case of two previous varieties. Further, its smaller assimilation of arabinose and its weak power of causing fermentation in fructose distinguish it from them. Moreover, its rather good growth in *α*-methylglucoside solution and its inability of fermenting lactose distinguish it from variety II.

VII. Assimilation of nitrogen compounds and difference between glycerin and cane-sugar as regards ester formation.

Substances.	Remarks.
Asparagin + glycerin ...	Forms almost the same film as variety I and II, except that the ring is thicker (10 days at 23-24°C). Ester flavour was absent, while in Hayduck's solution it was strong.
Peptone <sup>40</sup> + glycerin ...	The film was hardly perceptible (difference from the previous two varieties), with deposit but no flavour (10 days at 23-24°C). Ester flavour was also absent in pepton Hayduck's solution (after 23 days).
Ammoniumphosphate + glycerin	Forms a thin film, but no ring growth (difference from var. I and II). Growth was better in ammoniumphosphate Hayduck's solution (same as in var. I and II), in which ester flavour was also observable.
Potassiumnitrate + glycerin	Half-covered film was formed after 3 days at 22-23°C. Same as in variety I after 2 months.

Thus, the yeast behaves similarly towards nitrogen compounds, as the previous two but differs from them in certain details.

VIII. Proteolysis.

Stab culture.	Uniformly distributed.
After 40 days. No liquefaction takes place.	The liquefaction commences at the top.
After 56 days. About 1.5 c.m. from surface of gelatine layer dissolved.	About 1.5 c.m. from surface of gelatine layer liquefied.
After 70 days. The liquefied part doubled in comparing to the above duration.	The liquefied part increased to about five times against to the above period.

IX. Assimilation of amino-acids.

40). The ring formed by the 4 varieties in peptone (not asparagin) Hayduck solution was intensely yellowish brown in color while the ring formed in glycerin (not cane sugar) Hayduck solution was very slightly yellow.



Amino acids in original solution ..... 0.123% (as glycocoll).

„ ....after the growth ..... 0.018% ( „ ).

„ assimilated ..... 0.105% ( „ ).

The assimilation of amino-acids is greater than in variety II, but almost equal to that of var. I.

X. Optimum temperature for growth. It lies between 30° and 31° C.

XI. Death temperature. In diluted saké (water 50%) heating to 56-57° C for 30 minutes kills the yeast, but not in koji-extract, beer wort or yeast water; while 5 minutes heating in diluted saké to 57-58° brings about death but not in koji-extract.

XII. Limit of alcohol content for growth.

It forms a trace of film in koji-extract containing 10% of alcohol after 6 days at 27° C; while in koji-extract containing 15% of alcohol, there was no film or deposit. After this observation the culture was held at the room temperature of 17-20° C, and after 31 days there was a trace of deposit.

From the characters described above this yeast too belongs to the group of *Willia anomala*, but it is distinguishable from the other three varieties by its very long cell formed in the film, from var. I by its power of fermenting maltose and inability to ferment raffinose, from var. II by its greater assimilation of fructose, from var. I and II by the vigorous growth in maltose containing solutions, from var. II by not fermenting lactose, and moreover from var. I and II by the non-development of ester flavour or film in ammonium acetate solution (sol. I) devoid of ethylalcohol. As regards proteolysis it resembles var. IV, and behaves quite differently from var. I and II. As to the power of resistance against alcohol this variety is the weakest of the four varieties. The formation of fruit ester flavour by this yeast in ammonium lactate solution (sol. VII) is an important character distinguishing it from var. I and II.



**No. 4. *Willia anomala* Var. IV.**

I. Form and Size: (a) *Young culture*:—koji-extract culture at 23-25° C for 3 days. Filamental cells predominate:—curved cells ( $12.5 \times 3 \mu$ ;  $15 \times 3 \mu$ ), filamental cells ( $12.5 \times 4 \mu$ ,  $10 \times 4 \mu$ ). Round ( $5 \times 5 \mu$ ) or elliptical cells ( $2.5 \times 4 \mu$ ,  $7 \times 5 \mu$ ) are also found, but seldom. The presence of glycogen is not certain, and fat globules are not found. (b). *Old culture*: Surface culture on saké-agar during 4 months, from January to May. In the growth elliptical cells predominate ( $4 \times 3 \mu$ ), but long ( $6 \times 2.5 \mu$ ,  $20 \times 2.5 \mu$ ), round ( $4 \times 4 \mu$ ) or elliptical ones ( $5 \times 2.5 \mu$ ) are also found though rarely. Two hat shaped spores, are contained in a cell and some of the spores are outside the cell. In the surface culture of koji-extract agar at the room temperature for 27 days, the predominant cells are elliptical ( $5 \times 6$ ,  $5 \times 3$ ,  $5 \times 5 \mu$ ) and smaller elliptical cells ( $2 \times 2.5 \mu$ ) sousage shaped cells ( $11 \times 3 \mu$ ), or pear shaped cells ( $7 \times 4 \mu$ ) are found rarely.

II. Growth: The colony in the saké-agar *plate culture* (22 days at 25° C) is flat and almost same as in variety I, the only difference being that the marginal part is rather coarse and not feather. *Surface culture*. On koji-extract-agar (52 days at the room temperature) it forms a greyish white (as in var. II) and almost flat covering (in var III it was mesenteric). The growth on the thinner part of the medium subsequently becomes chalky white and the margin contains promycellium.

On koji-extract agar (3 months at the room temp.) the growth was similar to that of variety III, with however a lusterless surface. The covering on Moromi-agar was quite the same as in var III. On saké-gelatine (one month at 10-15° C) the covering was intensely brown (in the

41). The ledge of the hat is fairly well developed.

other three varieties it was white), and the central elevation was covered by many layers of the growths with radiations in the marginal part (as in var. III). The streamings of the marginal part were coarser than in var III.

*Fluid culture:* In beer wort (3 days at 23° C) it forms a thick film as in var III, but the deposit formed is the least of the four varieties; while fermentation is the most vigorous. In koji-extract (10° B, 16 days at 15-16° C) it forms a greyish white (different from var III), sharply folded (mesentery like) thick film which ascends the wall.

Deposit anw acetic—and fruit ester flavour present. A few of the film cells *stain red* with methylenblue. (80 days culture in koji-extract).

### III. Ester formation and its relation to carbon-source.

Solutions.	Conditions.	Remarks.
No. I ... ..	In test tube. 2 months at room temp.	Similar to var. I or II.
No. II... ..	Do.	Similar growth as in var. I or II.
No. II... ..	12 days at 25-27.5°C.	Same as var. III.
No. II .. ...	In sealed flask. 3 months at 23-25°C.	Ester flavour has disappeared already.
No. III ... ..	In test tube. 2 months at room temp.	Same as in var. III.
No. IV ... ..	14 days at 17-18°C.	Film formed was thinner than that of var. I, thereby distinguishable from var. II and III. Copious deposit formed with conspicuous fruit ester flavour.
No. V... ..	14 days at 17°C.	Film thinner than that of var. II and no spot as in var III. Ester flavour was perceivable.
No. V ... ..	In sealed flask. 2 months at 23-25°C.	Ester flavour was notable but no film formation.
No. VI ... ..	Do.	Forms almost the same film as var. II, and it was thicker than that of solution no IV, which did not contain amylalcohol, while this did.

Solutions.	Conditions.	Remarks.
No. VII ... ..	Do.	Forms a white but spotless film (differences from var. III). Ester flavour was copious, distinguishing this from var. II and III.
No. VIII ... ..	10 days at 25-26°C.	The film formed was almost same as in var. III and the fruit ester flavour was noticeable. Ester flavour was absent in var. I and II.
No. IX ... ..	4 days at 27.5°C.	The folds of film distinguishes this from var. II or III.

The utilization of acetate, butyrate, succinate and free succinic acid was also noted as carbon sources by this yeast. The mode of growth in these solutions distinguishes this variety from the other three. The formation of ester from ammonium acetate and ethylalcohol (Sol. No. II) or simply from ammonium acetate (solution I) took place as in var. I or II. Moreover, the ester formation from butyrate, succinate and lactate in presence of ethylalcohol (solution IV, V, and VIII.) was conspicuous, especially the production of fruit ester from lactate, as we have mentioned in var. III, has a special physiological interest.

IV. Spore formation: Two or four spores are formed in a cell on gypsum block at 27° C for 38 hours.

V. Fermentation products: Fusel oil, methyl alcohol, acetone and fruit esters were detected in the distillate of Koji-extract culture. Quantitative determinations gave the following results:—

Ethylalcohol .....	3.42 vol%.
Total esters .....	0.250 (as ethyl succinate)
Total acids .....	0.0182 (as succinic acid)

VI. Behavior towards carbohydrates.:

Substances.	Remarks.
Arabinose ... ..	Makes better growth than var. III and almost the same as var. I (3 days at 23.5-24°C).
Xylose ... ..	Very similar to var. I and III.
Fructose ... ..	The film was the thickest of the four varieties, but fermentation was almost absent (3 days at 23.5-24°C). The blue coloration obtained with Davy's test was the farthest of the four varieties.
Glucose ... ..	Forms white thin folded film after 4 days at 23.5-24°C. The folds were not observed in the three varieties mentioned above. The evolution of CO <sub>2</sub> -gas was vigorous.
Galactose ... ..	A very thin and almost transparent film was formed after 4 days at 23.5-24°C. Gave a very slight blue color with Davy's test after 15 days.
Cane-Sugar ... ..	Forms a white thin film, but the thickest of the four varieties, after 4 days at 23.5-24°C. A trace of folds was perceptible in the marginal part of the film, with an energetic evolution of CO <sub>2</sub> -gas.
Lactose ... ..	Similar to var. I, II or III (4 days at 23.5-24°C). Davy's reaction failed after 15 days.
Maltose ... ..	As in var. III, it forms a folded film (3 days at 23.5-24°C. Fruit ester flavour was conspicuous as in the other 3 varieties. Davy's reaction failed after 10 days.
Raffinose ... ..	A white film was formed after 3 days at 23.5-24°C. The film was thinner than that of var. II, 24°C. Davy's test for alcohol gave a negative result after 15 days.
Glycerin ... ..	Forms very thin and island like growth, after 3 days at 23.5-25°C.
$\alpha$ -methylglucoside ... ..	Half covered film was formed after 3 days at 23.5-24°C. The film was the thickest of the four varieties.

Thus, this variety also assimilates the above mentioned compounds as carbon-source, and is distinguished from the other three varieties by its worst growth in glycerin containing, by the formation of a folded film in solutions containing saccharose or maltose.

In regard to its fermenting property, this variety may be distin-

guished from var. I or II by not fermenting levulose and from var. II or III by not fermenting maltose.

VII. Assimilation of nitrogen compounds and difference between glycerin and saccharose as regards ester formation.

Substances.	Remarks.
Asparagin + glycerin ...	The film was almost equal to that of var. III, except as regarded the greater thickness the folded character of the film (10 days at 23-24°C). No ester flavour was perceptible as in the culture of Hayduck's solution.
Peptone + glycerin ...	The growth was almost equal except that the ring growth was thicker than in var. III (10 days at 23-24°C). No ester flavour was found, while in the culture of peptone-Hayduck's solution its formation was evident, though in traces.
Ammoniumphosphate glycerin. +	Develops like var. III (10 days at 23.5-24°C). Ester flavour was not developped as in the culture of ammonium phosphate Hayduck's solution.
K-nitrate + glycerin ...	Half covered film was formed (3 days at 22-23°C). After 2 months, there was formed a deposit, but there was no ester flavour; while in the culture of K-nitrate Hayduck's solution ester flavour was conspicuous with copious deposit of a yellow color.

Thus, this variety too assimilates asparagin, peptone, ammonium, and nitrate nitrogen. The non-formation of ester in glycerin-Hayduck's solution (changing nitrogen source as shown in above table) and its formation in Hayduck's solution or peptone; ammonium-phosphate—, potassiumnitrate Hayduck's solution, clearly shows that glycerin behaves quite differently from saccharose in regard to ester production.

#### VIII. Proteolysis.

Stab culture.	Uniformly distributed.
After 40 days. No liquefaction takes place.	Liquefaction commences at the top.
After 46 days. Liquefaction commences at the top of gelatine.	Liquefied part increased.
After 56 days. About 2.5 c.m. of gelatine layer from the top liquefied.	About 2 c.m. of gelatine layer from the top liquefied.
After 76 days. About 4 c.m. of gelatine layer from the top liquefied.	About 3.5 c.m. of gelatine layer from the top liquefied.

## IX. Assimilation of ammino-acids.

Amino-acids in original Koji-extract. . 0;1239, (as glycocoll)

Amino-acid after fermentation . . . . . 0'0104% (as glycocoll)

Amino acid assimilated . . . . . 0'1126% (as glycocoll)

X. Optimum temperature for growth: It lies between 30° and 31° C.

XI. Death temperature: In diluted saké (50% of water) or in beer wort heating to 56-57° C for 30 minutes kills the cell, but not in Koji-extract.

Death will ensue in diluted saké or koji-extract on heating it to 57-58° C for 5 minutes.

## XII. Limit of alcohol content for growth.

In koji-extract containig 10% of alcohol it forms a film and deposit after 7 days at 27° C, but there was no growth in koji-extract containing 15% of alcohol. The culture was subsequently held at the room temperature of 17-20° C, and a white deposit formed after 31 days, to which was added a film after 36 days.

Thus, this variety also belongs to the group of *Willia anomala*, and the main properties which distinguish it from the other three are the form of the colonies formed on saké-agar plate culture and the developement of a brown or greyish color in its growth in several media. Further, the filamental shape of the cells distinguishes this yeast from var. I and II.

The formation of ester by this variety in a lactate solution (solution No. VIII) devoid of carbohydrate, a property which it has in common with var. III, distinguishes it from var. I and II. The *want of fermenting power for maltose* differentiates it from var. III. The other three varieties are not killed by heating to 57-58° C for 5 minutes, which is sufficient for the death of this variety.

*The properties which distinguish our Willia anomala varieties from well known varieties:*

*They differ from our varieties.*

	Var. I.	Var. II.	Var. III.	Var. IV.
W. anomala. V. belgica. (1)	By not fermenting saccharose and absence of ester formation.			
Lindner's W. anomala from mazum. (2)	By not fermenting saccharose.			
			By fermenting fructose.	
Willia anomala Steu- ber. No. I. (3)	By yellow film.	By not fermenting maltose.		
		By not fermenting galactose and fermenting fructose.		
				By yellow film.
W. a. Steuber. No. II. (4)	By the absence of forming ester and not fermenting glucose or galactose.			
	By brownish rosy film.		By brownish rosy film.	
W. a. Steuber. No. III. (5)	By the want of ester formation and not fermenting galactose, glucose and saccharose.			
	By yellow film.		By yellow film.	
W. a. Steuber. No. IV. (6)	By absence of forming ester and not fermenting galactose, glucose and saccharose.			
	By yellow film.		By yellow film.	
		By not fermenting maltose.		
Saitō's W. ano- mala. (7)	By the opt. temp. 28°C, and quick liquefaction of gelatine:—14 days. Also by death temp.:—65°C (5 m. in Koji-extract) and by the higher % of alcohol produced. (5%).			
	By greysh white film.		By greysh white film.	
			By fermenting fructose.	
	By fermenting maltose.		By fermenting fructose.	
Fukumoto's ano- mala I. (8)	By fermenting maltose.		By fermenting fructose.	
			By fermenting maltose.	
	By less resistance agenst alcohol.			



	Var. I.	Var. II.	Var. III.	Var. IV.
	By less resistance against alcohol.			
Fukumoto's anomala II. (9)	By fermenting maltose.			By fermenting maltose.
			By fermenting fructose.	
	By the less resistance against alcohol and much production of alcohol. (4-920).			
Fukumoto's anomala III. (10)			By fermenting fructose.	
Lindner's anomala from Mazum. (11)	By fermenting maltose.			By fermenting maltose.
		By fermenting raffinose.		
	By an energetic fermentation of fructose solution.			
Zeidler's anomala. (12)		By fermenting raffinose.		
	By fermenting maltose.		By fermenting fructose.	
				By fermenting maltose.
Lindner's anomala from green malt. (13)	By fermenting maltose.	By fermenting raffinose.		By fermenting maltose.
		By fermenting raffinose.		
			By fermenting fructose.	
	By the less production of alcohol (0.9%) and duration of spore formation. (17.5-19 h. at 28°C).			
Hansen's anomala. (14)		By fermenting raffinose.		
	By the active fermentation of galactose solution.			
Lindner's anomala from American Beer. (15)		By fermenting raffinose.		
			By fermenting fructose.	
Kozai's anomala (16)	By the feeble fermentation of beer wort.			



	Var. I.	Var. II.	Var. III.	Var. IV.
Meissner's anomala. 4. (17)	By the higher % (4.06%) of alcohol formed.			
		By chalky white film.		By chalky white film.
Meissner's anomala 7. (18)	By the higher % (5.03%) of alcohol formed.			
	By the yellowish-white film.		By yellowish-white film.	
Meissner's anomala 40. (19)	By the higher % (5.03%) of alcohol formed.			
		By white film.		By white film.
Willia Wichmanni. (20)	By the formation of slimy growth.			

### Summaries of Part I.

From the properties described above, it is highly probable that our yeast are quite new varieties of *Willia anomala* with the exception of var I, which behaves very similarly towards carbohydrates as the variety of Linder's *Willia anomala* isolated from an American beer (India wharf.)

However, Lindner's variety causes an active fermentation in levulose and galactose solutions, whereas our var. I ferments the latter sugar very sparingly and the fermentation of the former sugar is doubtful.

It is very important and interesting that these varieties behave very

- (1), (2), (11), (12), (13), (15). P. Lindner: Wochenschr. Brauerei. 1900, 17. No. 49.-51.
- (3), (4), (5), (6). L. Steuber. Zeit. f. gesamntes Brauw. 1900. 23. 3.
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- (16). Cent. Bact. Paras. Abt. II. 1900. G. 400.
- (17), (18), (19). R. Meissner: Landw. Jahrb. 1901, 30. 497-580.
- (20). Zikes, cent. f. B. 1906. Abt. Bd. XVI. No. 416.-S. 97-111.

differently as to ester formation when shavings of *Crytomeria japonica* are added to the solutions; also the fact that they form esters from organic acid salts or free acids in the presence of alcohol or simply from organic acid salts (except var. II) in the absence of carbohydrates. Further, the formation of ester in common media, containing carbohydrates, is well known property of *Willia anomala*. So, these varieties *form esters not only from carbohydrates, but also from preexisting alcohol and organic acids*. The fact that variety III grows better in alcohol containing the tions than in those without alcohol, in the absence of carbohydrates, shows well that *this variety also assimilates alcohol*:— a property distinguishing it from the common *mycederma* yeast, which is simply a destroyer of alcohol.

A copious evolution of the fruit ester flavour in *ammonium buyrate* containing solution *explains the role of this salt in saké brewing*<sup>42</sup>.

As regards the difference between carbohydrates and glycerin for ester formation, it is worth noticing that the formation of ester is always absent in the cultures of glycerin Hayduck's solution, whatever may be the source of nitrogen compounds.

The great assimilability of aminoacids by our yeast, is an important property for the *aging or after ripening of saké*; for common saké yeast *saccharomyces saké*<sup>43</sup> assimilates the acids moderately as compared with our *Willia anomala* varieties, e.g. *Sacch. saké*. B. 21 of Oji Saké Brewing Institute assimilates only 0.059% of the acids from a solution containing 0.123% while our *Willia* varieties assimilate 0.116-0.097% of the acids according to the varieties.

(42). cf. Kurono's article of this Journal.

(43). cf. Takahashi and Yamamoto's article. The formation and assimilation of aminoacids by the different varieties of yeast. P. 279 of this Journal.

## PART II.

## Application of the Aging Yeast.

The first experiment (August 1908).

To seven flasks, each containing 700 c. c. of newly prepared young saké, were heated for 15 minutes to 55° C and after addition of a sterilised piece of *Cryptomeria japonica* were added the varieties of our yeast, one to each flask, and kept at the room temperature. After 5 days the result was as follows:—

Number of Samples	Yeast	Quality.
I.	...	A very slight change occurred.
II.	Var. I.	Made the best change in palatableness and characteristic flavor of young saké disappeared.
III.	Var. I.	Do.
IV.	Var. II.	Almost same as no. I.
V.	Var. II.	Do.
VI.	Var. III.	Better than no IV. or V
VII.	Var. IV.	Do.

Thus, the ripening of saké was accelerated by the addition of the yeast.

Second experiment (December 1908).

By the first experiment the role of our *Willia anomala* was placed beyond doubt, therefore in this case some of the flasks were kept at 20-25° C, while others were kept at the room temperature below 20° C, for the purpose of determining the influence of temperature during aging. The flasks containing young saké were heated for 20 minutes to 60-65° C,

and the yeast was added as in the first experiment, not omitting the addition of a piece of *Cryptomeria japonica*.

I. Series, where the duration of experiment was 2 weeks.

No. of Samples.	Yeast	Temp.	Quality.
I.	...	At 20-25°C.	Inferior to No XIII.
III.	...	Room temp.	The worst of this series.
IV.	...	Room temp.	Do.
V.	Var. I.	At 20-25°C.	Stand next of No. XVIII, but better than No. V.
X.	Var. II.	Do.	Stand next of no. V.
XIII.	Var. III.	Do.	Almost same as No. X.
XVIII.	Var. IV.	Do.	<i>The best of the first series.</i>

II. Series: The duration of aging was 3 weeks at 20-25° C.

No. of Samples.	Yeast	Quality.
II.	...	The worst of this series.
VI.	Var. I.	Stand next to No. XI, and same as No. XIV. or XIX.
XI.	Var. II.	<i>The best of this series.</i>
XIV.	Var. III.	Same as No. XI, or No. XIX.
XIX.	Var. IV.	Same as No. VI. or XIV.

III. Series: The duration of aging was about one month at 20-25° C.

No. of Samples.	Yeast.	Quality.
VIII.	Var. I.	<i>The best of this series.</i>
XII.	Var. II.	Stand next, in quality, to No. VIII, with a somewhat sweet taste.
XVI.	Var. III.	Gave unpleasant flavour and was the worst of this series
XX.	Var. IV.	Almost same as No. XII, but with a trace of bitter taste.

As the first series shows, the temperature of the fluid has a large influence upon aging, and moreover the duration of aging is different according to the varieties of yeast, as shown by the other series of this experiment.

Var I. needs the longest time and var IV. the shortest, while var II stands in the middle.

Third experiment (January 1909).

In this experiment lactate—or butyrate of ammonium of which the latter was the best substance for ester formation, was added to the saké before the experiment. The temperature was 20-25° C and the duration 2 weeks.

No. of Samples.	Yeast.	Organic Salt.	Remarks.
I.	...	...	Almost same as No. III, IV, or V.
II.	Var. I.	...	The best of all.
III.	Var. II.	...	Almost same as No. I.
IV.	Var. III.	...	Do.
V.	Var. IV.	...	Do.
VI.	Var. I.	0.4 c.c. butyrate to 100 c.c. Saké.	Inferior in quality, with flavor of butyric acid.
VII.	Var. II.	Do.	Do

No. of Samples.	Yeast.	Organic Salt.	Remarks.
VIII.	...	0.2 c.c. of butyrate to 100 c.c. Saké.	Inferior in quality, with flavor of butyric acid.
IX.	...	Do.	Do.
X.	Var. I.	0.2 c.c. of lactate to 100 c.c. Saké.	Do.
XI.	Var. III.	Do.	Do.
XII.	Var. III.	Do.	Do.
XIII.	Var. IV.	Do.	Do.
XIV.	Var. I.	0.2 c.c. of both butyrate and lactate in 100 c.c. Saké.	Do.
XV.	Var. II.	Do.	Do.
XVI.	Var. III.	Do.	Do.
XVII.	Var. IV.	Do.	Do.

In the culture of the four varieties of our *Willia* in butyrate or lactate containing solution, the formation of ester was conspicuous; while in this third experiment butyric acid was formed but no ester. The cause of this is attributable with reason to the excess of these salts, so that the further experiments were made.

Fourth experiment (February 1909).

The duration of experiment was one month at 20° C.

No. of Samples.	Yeast	Salts.	Remarks.
I.	...	...	Medium quality, showing a little change for ripening.
II.	Var. I.	...	Better than No. I.
III.	Var. II.	...	Do.
IV.	Var. III.	...	Do.
V.	Var. IV.	...	Better than No. II, III, or IV.
VI.	Var. I.	0.01 c.c. of butyrate to 100 c.c. Saké.	Palatable.
VII.	Var. II.	Do.	Do.
VIII.	Var. III.	Do.	Bad odor.
IX.	Var. IV.	Do.	Do.
X.	Var. I.	0.005 c.c. of butyrate to 100 c.c. Saké.	Medium quality as No. I.
XI.	Var. II.	Do.	Do.
XII.	Var. III.	Do.	Do.
XIII.	Var. IV.	Do.	Do.
XIV.	...	0.01 c.c. of butyrate to 100 c.c. Saké.	Both the flavour and taste were not good.
XV.	...	0.005 c.c. of butyrate to 100 c.c. Saké.	Stands next to No. XVII.
XVI.	...	0.0025 c.c. of butyrate to 100 c.c. Saké.	Do.
XVII.	...	0.0005 c.c. of butyrate to 100 c.c. Saké.	<i>The best of all.</i>

Fifth experiment (June 1909). 2 weeks at near 20° C.

No. of samples.	Wood <sup>44</sup> piece.	Yeast.	C.c. of butyrate to 100c.c. of Saké.	Remarks.
I	...	...	...	The flavour of young saké still remains.
II	...	...	0.0001 c.c.	The flavour of altered saké.
III	+	...	...	Trace of the flavour of young saké still remains.
IV	+	...	0.0001 c.c.	Do, but a little better.
V	+	Var. I	...	Trace of the flavour of young saké still remains.
VI	+	Var. II	...	Do.
VII	+	Var. III	...	Do.
VIII	+	Var. IV	...	Change for ripening sufficient to make a palatable saké.
IX	+	Var. I	0.001 c.c.	Almost same as no. VI and VII.
X	+	Var. II	Do.	Change for ripening sufficient to make a palatable saké.
XI	+	Var. III	Do.	The taste was best of all samples, flavour in less good somewhat improved.
XII	+	Var. IV	Do.	Do.
XIII	+	Var. I	0.0002 c.c.	Do.
XIV	+	Var. II	Do.	Do.
XV	+	Var. III	Do.	Do.
XVI	+	Var. IV	Do.	Do.
XVII	+	Var. I	0.0001 c.c.	Do.
XVIII	+	Var. II	Do.	Do.
XIX	+	Var. III	Do.	Do.
XX	+	Var. IV	Do.	Change for ripening was sufficient.

This experiment proves the influence of *Cryptomeria japonica* for the aging process as mentioned in the first part.

Sixth experiment (June 1909).

The fifth experiment, in which the storage was for 2 weeks, showed



that a suitable quantity of ammonium butyrate was favourable for the aging. In this experiment the storage time was therefore prolonged to almost one month at 20-27° C, other conditions being identical.

No. of Samples.	Wood piece.	Yeast.	C.c. of butyrate to 100 c.c. of Saké.	Remarks.
I	+	...	...	The flavour of young saké still remained.
II	+	Var. II	...	Change of ripening further advanced than in no. I.
III	+	Var. III	...	The flavour and taste were not good.
IV	+	Var. IV	...	Do.
V	+	Var. II	0.0001 c.c.	Almost equal no. II.
VI	+	Var. IV	Do.	Palatable.
VII	+	Var. II	0.001 c.c.	Do.
VIII	+	Var. III	Do.	Very Palatable.
IX	+	Var. IV	Do.	Palatable.
X	+	...	0.0001 c.c.	Almost same as no. IX.
XI	+	...	0.001 c.c.	Palatable.

Thus, the suitable quantity of butyrate for aging with yeast is seen to be 0.00/c.c. to 100 c.c. of saké, and one month storage was too long especially for var III and IV. when yeast was simply added, as we observed in the second experiment (var. IV. needs the shortest storage time for aging). Moreover, *simple addition of butyrate was effective for aging.*

Seventh experiment (January 1910): 10 days at 17-22° C.

From the sixth experiment the role of our *Willia anomala*, by itself or with the addition of butyrate, was positively ascertained, therefore in this case eight wooden tubs, made of *Cryptomeria japonica*, of about 36 L. capacity were used instead of glass flasks.

No. of tubs.	Yeast.	Butyrate or others.	Remarks.
I	...	...	Better than no. VII.
II	Var. II	...	Very palatable.
III	Var. III	...	Do.
IV	Var. IV	...	Better than no. V.
V	Var. II, III and IV	0.0001 c.c.	Better than no. I.
VI	Do.	...	Almost same in palatableness as no. I.
VII	Do.	200 c.c. of nutrient fluid over the yeast	The worst in quality, being thick in color.
VIII	...	0.0001 c.c. of butyrate to 100 c.c. of saké	Better than no. IV.

The chemical composition of the saké after the experiment was as follows:—

	Original.	Tub. I.	II.	III.	IV	V.	VI.	VII.	VIII.
Sp. gr. ... ..	0.9930	0.9931	0.9923	0.9929	0.9928	0.9930	0.9928	0.9928	0.9928

100 c.c. of saké contains in grams of.

Alcohol (vol.)... ..	17.60	16.98	17.90	17.16	17.00	17.00	17.00	17.10	17.30
Extractive matters...	3.8056	3.9087	3.9568	3.9792	3.9170	3.9552	3.9384	3.9556	3.9384
Total acid ... ..	0.1770	0.1770	0.1770	0.1770	0.1770	0.1770	0.1770	0.1770	0.1770
Volatile acid ... ..	0.0360	0.0360	0.0300	0.0360	0.0360	0.0360	0.0360	0.0360	0.0360
Nonvol. acid ... ..	0.1416	0.1416	0.1475	0.1416	0.1416	0.1416	0.1416	0.1416	0.1416
Ash ... ..	0.0440	0.0488	0.0488	0.0480	0.0444	0.0472	0.0440	0.0468	0.0464
Glycerin ... ..	1.1840	1.1524	1.1460	1.1244	1.1028	1.1046	1.0866	1.1088	1.1440
Glucose ... ..	1.1984	1.1842	1.1460	1.2048	1.1874	1.1608	1.1252	1.1460	1.1660
Dextrin ... ..	0.5900	0.5678	0.5480	0.6678	0.5682	0.5272	0.5873	0.5794	0.5776
Crude protein... ..	0.7158	0.7140	0.7140	0.7175	0.7105	0.7125	0.7105	0.7105	0.7095
Protein (Stutzer's method)	0.0403	0.0401	0.0385	0.0420	0.0455	0.0402	0.0420	0.0428	0.0455

	Original.	Tub. I.	II.	III.	IV.	V.	VI.	VII.	VIII.
Fusel oil (after Takahashi)	0-1500	0-1500	0-1500	0-1500	0-1500	0-1500	0-1500	0-1500	0-1500
Aldehyde (as acet-aldehyde)	0-0159	0-0035	0-0088	...	0-0070	...	0-0088	0-0086	...
Furfural ... ..	0-0010	0-0010	0-0007	0-0008	0-0007	0-0009	0-0007	0-0009	0-0005
Amino acids (as glyocoll) ...	... c.c.	0-2126	0-1890	...	...	0-1939	0-2010	...	...
Degree of } {Iod. <sup>45</sup> coloration } {K. Bich.	0-83 1-2	1-1 1-4	1-0 1-4	1-0 1-0	1-0 1-4	1-0 1-4	1-1 1-4	1-3 1-4	1-4 1-1

Thus, there is an increase of ethylalcohol, aldehyde, and protein (in a few examples) in the tubs, when our *Willia anomala* was added. The increase of ethylalcohol is evidently the result of alcoholic fermentation due to the yeast and the aldehyde must also be ascribed to the same action; while an increase in protein contents in certain tubs (No. IV, VII, VIII) is explained by the presence of suspended yeast cells in these samples. The decrease of furfural, glycerin, and amino acids was observable in all the tubs except in control tub. The decrease of the last two substances is a settled matter from our *Willia anomala*, but why the decrease of furfural still needs explanation. Perhaps Will's<sup>46</sup> and Lintner's<sup>47</sup> observation of the decrease of furfural in wort by the growth of yeast furnishes the only basis for the explanation of our case. Moreover, the increase of acetic and another esters (not given in the table) and the disappearance of the sharp flavor of young saké are decidedly favorable factors for aging.

#### Eighth experiment (January 1910).

(45). The Iodine solution contained 1-27 gr. of iodine and 1-8 grams of KI. in one litre of water. The K-bichromate solution contained 1-94 grams of the salt in one litre of water.

(46). Zeitschrift f. gesamtes Brauw. 1902. 25. 39.

(47). Lintner. (Brewer's Jouru. 1910. No. 11. S. 535). He ascribes the decrease of furfural to the formation of thiofural.

By this experiment the esters, formed by the mixed (Var. I, II, III) culture in Karlsberg's flask containing Koji-extract, were passed through young saké twice every day:—each passage lasting 30 minutes. After 10 days the sharp flavour of young saké disappeared and gave place to the flavour of fruit esters.

### Conclusion.

From the result of the above experiments we must conclude that during the after ripening or the aging of saké, there must be present certain varieties of *Willia anomala*, which produce definite changes in the composition of young saké, and that the artificial addition of this yeast to young saké accelerates the ripening, producing well aged saké in a comparatively short time.

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#### Explanation of Figures.

##### Plate XII.

Colonies in saké-agar plate culture.

Fig. 1, A = Variety I.

Fig. 2, B = Variety II.

Fig. 3, C = Variety III.

Fig. 4, D = Variety IV.

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## The Quantity of Amino-acids and its Relation to the Quality of Saké

BY

T. Takahashi and H. Satō.

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It is a fact beyond dispute that chemical composition of saké has a certain relation to its quality, but the chemical reserches made so far are insufficient to determine it precisely. One<sup>1</sup> of us has a certain relation to few exceptions, the quantity of fusel oil in saké has a certain relation to its quality, the better the saké the less it contains fusel oil.

No other fact is known which appeared to us to have any constant relation to the quality of saké, so we have made some reserches on the amino acids contained in saké according to Sørensen's<sup>2</sup> method the results were as follows:—

- (1). Takahashi. The Journal of the Tokyo chemical society Band 26, vol. VIII.
- (2). Sørensen's method: deut. chem. Gesel 1908. Bd. I. S. 143-144.

Name of brewers.	Quality of saké.	Amino acids (as glycocoll) %.
S. Ōyagi ... ..	Superior	0.174
T. Miura ... ..	"	0.178
S. Kimura ... ..	"	0.210
S. Tatsuumi ... ..	"	0.187
T. Saito ... ..	Best, 1st class	0.163
T. Ōkura ... ..	"	0.171
S. Ōyagi ... ..	"	0.183
M. Kawameto ... ..	"	0.174
J. Matsumoto ... ..	"	0.186
Y. Sugamoto ... ..	"	0.187
T. Yamazaki ... ..	"	0.189
Aké Company ... ..	"	0.195
G. Koezuka ... ..	"	0.186
B. Osabe ... ..	"	0.218
J. Hanaki ... ..	"	0.203
Eigashima Company ... ..	"	0.192
T. Yamamura ... ..	"	0.214
G. Tsuga ... ..	"	0.172
T. Washiwa ... ..	"	0.167
T. Fujii ... ..	"	0.155
J. Kano ... ..	"	0.192
K. Kimura ... ..	"	0.210
S. Imanari ... ..	"	0.254
J. Takanashi ... ..	"	0.234
M. Kimura ... ..	"	0.245
J. Kutsuzawa ... ..	"	0.272
M. Hara ... ..	"	0.161
" ... ..	"	0.167
S. Isota ... ..	"	0.230
K. Shimo ... ..	"	0.185
S. Iwoya ... ..	"	0.196



Name of brewers.	Quality of saké.	Amino acids (as glycocoll) %.
J. Shiwozaki ... ..	Best	0.171
O. Takata ... ..	..	0.167
I. Katō ... ..	..	0.166
K. Matsuoka ... ..	..	0.233
T. Shimo ... ..	..	0.187
K. Shimo ... ..	..	0.209
S. Kimura... ..	..	0.180
K. Ihara ... ..	..	0.178
K. Ōbayashi ... ..	..	0.220
S. Kimura... ..	..	0.207
M. Makibayashi ... ..	..	0.221
Y. Fujii ... ..	..	0.172
N. Ōtō ... ..	..	0.208
T. Miura ... ..	..	0.227
H. Miyahara ... ..	..	0.201
M. Takashiige ... ..	..	0.217
G. Mitsuishi ... ..	..	0.247
T. Murakami ... ..	..	0.192
T. Taketsuru ... ..	..	0.159
T. Morishita ... ..	..	0.180
” ... ..	..	0.172
T. Taketsuru ... ..	..	0.172
H. Shima ... ..	..	0.166
R. Matsui ... ..	..	0.149
S. Yoshioka ... ..	..	0.238
S. Ishii ... ..	..	0.172
I. Shigeta ... ..	..	0.250
R. Horie ... ..	..	0.247
S. Nishimura ... ..	..	0.209
T. Hirano... ..	..	0.226
Z. Ikeda ... ..	..	0.254

Name of brewers.	Quality of saké.	Amino-acids (as glycoll) %.
K. Ishii ... ..	Best	0.177
K. Matsuo... ..	"	0.205
T. Kohiyama ... ..	Better, 2nd class	0.296
T. Sasashima ... ..	"	0.192
K. Yamawaki ... ..	"	0.230
I. Tomiyasu ... ..	"	0.214
Y. Tsunono ... ..	Good, 3rd class	0.279
J. Okamura ... ..	"	0.202
R. Tada ... ..	"	0.263
T. Nakano ... ..	"	0.291
J. Tomono ... ..	"	0.272
M. Kawai ... ..	"	0.246
I. Hasegawa ... ..	"	0.331
Y. Hashimoto ... ..	Inferior	0.263
J. Yokozeki ... ..	"	0.250
M. Mutō ... ..	"	0.286
R. Nishijo... ..	"	0.214
R. Hirano... ..	"	0.200
G. Nate ... ..	"	0.273
T. Shimamura ... ..	"	0.243
R. Tamai ... ..	"	0.241
F. Nakayama ... ..	"	0.349
K. Sakurai ... ..	"	0.239
K. Saihara ... ..	"	0.218
I. Watanabe ... ..	"	0.265
S. Hirai ... ..	"	0.297
S. Ueda ... ..	"	0.272
G. Kabachi ... ..	"	0.250
U. Mitsuyasu ... ..	"	0.275
S. Nakamura ... ..	"	0.316
E. Takahashi ... ..	"	0.247

Name of brewers.	Quality of saké.	Amino-acids (as glycocoll) %.
K. Takano ... ..	Inferior	0.277
J. Shiwoiri ... ..	"	0.245
T. Sakai ... ..	"	0.286
N. Ueda ... ..	"	0.393
H. Takata... ..	"	0.324
T. Kamaya ... ..	"	0.238
T. Kitai ... ..	"	0.273
S. Satō ... ..	"	0.199
G. Hirata ... ..	"	0.324
K. Murata... ..	"	0.261
H. Hirai ... ..	"	0.183
S. Iikura ... ..	"	0.261
J. Koike ... ..	"	0.337
J. Kutsusawa ... ..	"	0.255
S. Murata ... ..	"	0.366
S. Shiramizu ... ..	"	0.264
T. Ma'sumura ... ..	"	0.222
K. Tanaka ... ..	"	0.253
K. Itō ... ..	"	0.212
K. Fujishima ... ..	"	0.264

In 64 samples including 4 superior and 60 first class saké we have.

Maximum ..... 0.272% of amino acids.

Minimum ..... 0.149% of amino acids.

Average ..... 0.197% of amino acids.

In 4 samples of the second class we have:—

Maximum ..... 0.296% of amino acids.

Minimum ..... 0.192% of amino acids.

Average ..... 0.233% of amino acids.

And in 46 samples including 7 of the third and 39 of the inferior class we have;—

Maximum .....	0.393% of amino-acids.
Minimum .....	0.183% of amino-acids.
Average .....	0.268% of amino-acids.

*Thus, on an average better saké contains less amino-acids than the inferior ones.*

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**The Assimilation and Formation of Amino-acids  
by *Saccharomyces Saké* and Other  
Yeast Varieties.**

BY

**T. Takahashi and T. Yamamoto.**

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As a decomposition product of the protein matters of yeast cells by self-fermentation, leucine was found by Liebig<sup>1</sup> tyrosin by Béchamp<sup>2</sup>. Schützenberger mentions still another nitrogenous compound<sup>3</sup> as a product of self-fermentation. Recently Boullanger, Beijerinck, Artari, Wehmer and especially Will<sup>4</sup> made laborious researches on the proteolysis of yeast cultur. Also, Salkowski<sup>4</sup>, Effront<sup>5</sup> and Rettger<sup>6</sup> and many others followed with their studies on self-digestion, or autolysis and a new branch of mycology has been established. According to Geret and Hahn the nitrogenous matter of the pressed juice of yeast, decomposes into many amino-acids, of which about 30% consists of di-amino-acids or bases and 70% of mono-amino-acids. Recently, Kutscher and Lohman<sup>7</sup> analysed the self-fermentation products of beer yeast with Kossel-Kutscher's method and found histidin, Iysin, arginin, leucin, tyrosin, aspartic acid, ammonia and many other purin derivatives.

1. Sitzungsber. d. Kgl. bayr. Akad. d. Wiss. in München. 1868 u. 1869.

2. Compt. rend. de l'Ac. 1872. Bd. 74. S. 184

3. Alloxanbases and purin derivatives.

4. Zeit. f. phys. chem. 1889. Bd. 13. S. 506.

5, 6. Brewer's Journ. 1909. P. 540.

7. Zeit. f. phys. chem. 1903. Bd. 39. S. 159 u. 313.

Similar decompositions of protein matters take place in the nutrient media outside the yeast cells.

On the assimilation of amido-nitrogen by yeast, Wahl and Hantke<sup>8</sup> found in the culture of wort, that the assimilation of amido-nitrogen is very conspicuous as compared with that of peptone or protein nitrogen. According to P. Petiti's<sup>9</sup> observation the surface yeast cells consume more than double the amido-nitrogen (asparagin) than the bottom cells do. R. Kusserow<sup>10</sup> too arrived at the conclusion that the decomposition products of the protein matters are assimilated better than undecomposed proteins. Recently, Stockhausen<sup>11</sup> made researches on the assimilation of the decomposition products<sup>12</sup> during the self-digestion of beer yeast, and found that the assimilability of these decomposition products differed according to the varieties of yeast.

About saké yeast, K. Kurono<sup>13</sup> found that all nitrogenous substances used in his experiment were assimilated equally well, except tyrosin, cystin, nitrate and nitrite.

It has seemed to the authors that the assimilability of amino-acids differs somewhat according to the varieties of saké yeast; for the quantities of amino-acids of saké differs evidently in accordance with its quality as described in another paper. The experiment was carried on with 100 c.c. of sterilized Koji-extract (10° B) in Erlenmeyer's flask and after the

8. American Brewer's Review. 1894. B. 7. S. 492.

9. Compt. Rend. T. CXXIV. 1897. P. 93.

10. Zeits. für Spiritind. 1897. Bd. 20. S. 97.

11. Jahrb. der Versuch. u. Lehr. f. Brau. in Berlin. 1908. 11. S. 673.

12. The compounds used in his experiment were;—Leucine, tyrosin, histidin, arginin, adenin, hypoxanthin, guanidin, lysin, cholin, uracil, glutamic acid, aspartic acid, tetramethylen-diamin, and ammonia. Further, thymin and asparagin.

13. Journal of the Scient. Agricul. Socie. No. 91. 1910 also this journal. He used as nitrogen-source: Glycocoll, alanin, leucin, aspartic acid, (free and neutral), asparagin, tyrosin, cystin, glutaminic acid, arginin, histidin, phosphate and carbonate of ammonium, nitrate-nitrit of K. The variety of yeast used in his experiment was B. No. 25. used in our experiment.

addition of yeast it was kept at 25° C for 7 days. During this time the fluid was shaken once every day. For one portion of the clear fermented fluid the quantity of amino-acids was determined according to Sørensen's method<sup>14</sup>. Another portion of the fluid (50 c.c.) was distilled and for it the quantity of fusel oil was determined after T. Takahashi's method<sup>15</sup>. The results are given below:—

(Original koji-extract contained 0.148% of amino-acids).

Varieties of Yeast.		Amino-acids (as glycoll) %.		Fusel oil %.	Total acid %. (as succinic acid.)
		Remained.	Consumed or formed.		
A	I	0.084	0.064	Ca. 0.0125 less	0.0556
„	II	0.059	0.099	„ 0.0125 more	0.0592
„	III	0.099	0.049	„ 0.0125 less	0.0644
„	IV	0.066	0.082	„ 0.0125	0.0645
„	V	0.083	0.065	„ 0.0125	0.0404
„	VI	0.074	0.074	„ 0.0125 less	0.1068
„	VII	0.101	0.047	„ 0.0125 „	0.0488
„	VIII	0.087	0.069	„ 0.0125	0.0580
„	IX	0.318	* 0.170	„ 0.0125 more	0.0556
„	X	0.087	0.069	„ 0.0125 „	0.0528
„	XIII	0.072	0.076	„ 0.0125 less	0.0716
„	XIV	0.209	* 0.061	„ 0.0125	0.0672
„	XV	0.109	0.039	„ 0.0125 less	0.0891
„	XVI	0.120	0.028	„ 0.0125 „	0.0952
„	XVII	0.120	0.028	„ 0.0125 „	0.1193
„	XVIII	0.068	0.080	„ 0.0125 „	0.0921
„	XIX	0.133	0.015	„ 0.0125 „	0.0388
„	XX	0.112	0.036	„ 0.0125 „	0.0650
„	XXI	0.224	* 0.076	„ 0.0125 „	0.1085

14. Chem. cent. Bd. I. 1908. S. 143-144.

15. Bulletin of the College of Agric. Tokyo Imp. Univ. Vol. VI. No. 4. P. 438.

Varieties of Yeast.	Amino-acids (as glycoll) %.		Fusel oil %.	Total acid %. (as succinic acid.)
	Remained.	Consumed or formed.		
" XXII	0.158	* 0.010	Ca. 0.0125 "	0.1428
" XXIII	0.200	* 0.052	" 0.0125 "	0.0913
" XXIV	0.178	* 0.030	" 0.0125 "	0.0654
" XXV	0.086	0.062	" 0.0125 "	0.0775
" XXVI	0.053	0.095	" 0.0125 "	0.0351
" XXVII	0.078	0.070	" 0.0125 "	0.0121
" XXVIII	0.118	0.030	...	0.1026
" XXIX	0.038	0.110	" 0.0125 less	0.0097
" XXX	0.079	0.079	" 0.0250	0.0454
" XXXI	0.154	* 0.003	Amyl ester	0.0589
" XXXII	0.034	0.043	" 0.0125	0.0324
" XXXV	0.143	0.004	" 0.0125	0.0454
" XXXVI	0.085	0.062	" 0.0125 less	0.0490
B. I	0.117	0.030	" 0.0250	0.1220
" II	0.088	0.059	" 0.0125 less	0.0887
" III	0.058	0.089	" 0.0125 "	0.0430
" IV	0.059	0.088	" 0.0250 "	0.0610
" V	0.118	0.030	" 0.0250	0.0406
" VI	0.041	0.106	" 0.0250 less	0.0321
" VII	0.119	0.027	" 0.0125	0.0407
" VIII	0.076	0.070	" 0.0125 less	0.0528
" IX	0.059	0.087	" 0.0125 "	0.0436
" X	0.070	0.077	" 0.0125 "	0.0409
" XI	0.053	0.087	" 0.0125 "	0.0379
" XII	0.053	0.087	" 0.0500	0.0168
" XIII	0.082	0.065	" 0.0125	0.0407
" XIV	0.059	0.087	" 0.0250	0.0321
" XV	0.082	0.065	" 0.0250	0.0406
" XVI	0.053	0.087	" 0.0250	0.0406



Varieties of Yeast.	Amino acids (as glycooll) %.		Fusel oil %.	Total acid. % (as succinic acid).
	Remained.	Consumed or formed.		
„ XVII	0.065	0.083	„ 0.0125	0.0350
„ XVIII	0.041	0.106	„ 0.0125	0.0291
„ XIX	0.129	0.017	„ 0.0250	0.0032
„ XX	0.053	0.095	„ 0.0125	0.0236
„ XXI	0.100	0.047	„ 0.0250	0.0089
„ XXII	0.076	0.071	„ 0.0250	0.0238
„ XXIII	0.241	* 0.033	„ 0.0250 less	0.0442
„ XXIV	0.065	0.083	„ 0.0125	0.0292
„ XXV	0.038	0.059	„ 0.0125	0.0118
„ XXVI	0.100	0.047	„ 0.0125	0.0104
„ XXVII	0.076	0.071	„ 0.0125	0.0089
„ XXVIII	0.059	0.089	„ 0.0250 less	0.0349
„ XXIX	0.094	0.053	„ 0.0250	0.0239
„ XXX	0.029	0.118	„ 0.0250 less	0.0108

\* indicates formation.

As second series of experiments was made with beer yeast and other well known varieties, with the following results:—

Yeast varieties.	Amino-acids <sup>1</sup> %.		Fusel oil.
	Remained.	Consumed or formed.	
Champagne yeast ... ..	0.112	0.064	Plenty
Tarula (red var.) ... ..	0.206	+0.030	Medium
Surface beer yeast (Holland) ...	0.123	0.53	„
Bottom beer yeast (Munich) ...	0.023	0.153	Least
Shiz. Sacch. Pombe ... ..	0.153	0.023	Medium
Wine yeast (steinberg) ... ..	0.147	0.069	Plenty
Willia anomala Hansen ... ..	0.057	0.119	Medium

16. In original koji-extract there was 0.176% of amino-acids.

Thus, the quantity of the amino-acids assimilated varies according to the varieties of saké-yeast;—from 0.064%-0.004%. Moreover, with certain varieties there was an *increase of the amino-acids*, the case of A. No. 9 being an extreme example of these varieties:—the increase being 0.170%, or more than double the original quantity.

The increase of amino-acids corresponded with the intensity of proteolysis in A. No. 9, No. 21 and B. 23 where the proteolysis was most intense, but in A. 30, B. 8, 16, 24, where the proteolysis was also energetic, there was no increase of amino-acids in the fluid.

In general, the culture in which the consumption of amino-acids was large produced plenty of fusel oil, but there are exceptions: e.g. comparing A. 13 and 9 we have:—

	Amino-acids %.		Fusel oil.
	Remained.	Consumed or formed.	
A. 13 ... ..	0.073	-0.0760	Ca. 0.0125% less
A. 9 ... ..	0.3188	+0.0170	Ca. 0.0125% more

From this example, it is evident that the proteolytic enzyme acts more efficiently when there is a sufficient quantity of amino-acids as the nutriment of the yeast. Further, in other cases as A. 29 and 35 we have:—

	Amino-acids %.		Fusel oil %.
	Remained.	Consumed.	
A. 29 ... ..	0.0380	0.110	0.0125 less
A. 35 ... ..	0.1434	0.0036	0.0125

The quantity of fusel oil formed is altogether inverse to the quantity of amino-acids consumed. A similar phenomenon will be observed too in other varieties of yeast: e.g. champagne yeast or wine yeast forms plenty

of fusel oil, the consumption of amino-acids in this case being half that of the bottom beer yeast, which produces the least quantity of fusel oil. As to the cause of this inversion, we could not make it out; but perhaps it may be due to formation of a comparatively larger quantity of some intermediate products, such as addelyde or some derivatives of amylalcohol, such as esters; or to lesser assimilability of leucine as compared with the other amino-acids, or to the assimilability of leucine as such without the formation of fusel oil (A. 29.) At present we are not in a position to clear up this point.

### Conclusion.

I. The assimilability of amino-acids differs widely according to the varieties of yeast, and we must select, especially in saké-brewing, such yeast as consumes the greatest quantity of amino-acids and produces the least quantity of fusel oil.

II. In the cultures of certain varieties of saké-yeast and *Torula* (red var.), there was an increase of amino-acids inspite of the formation of fusel oil.

III. The quantity of acids formed in the culture has no relation to the other products.

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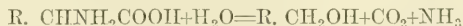
## On the Formation of Fusel oil by Saké Yeast.

BY

K. KURONO.

---

The formation of fusel oil during alcoholic fermentation has long been the subject of discussion, both from the chemical and the biological point of view but a satisfactory solution has only been given by the recent investigation of Felix Ehrlich<sup>1</sup>. According to him, fusel oil is chiefly formed from leucine, by the action of yeast, the chemical change being expressed in the following general equation.



Thus from d, l and inactive leucine, the corresponding amyl-alcohol i.e. fusel oil may be formed. The ammonia produced in this process will serve directly for the formation of proteins in the new cells. Although Ehrlich's view has been repeatedly confirmed by various authors and many works have been carried out along this line, yet there remain still many questions to be settled. Thus for instance, different kinds of yeast must produce different amounts of fusel oil under the same condition, and moreover there is no doubt that various factors exert great influence upon the production of fusel oil. All these points ought to be studied thoroughly.

As Japanese saké is especially rich in fusel oil and its elimination is one of the most important questions for the brewers, I have repeated

1. Berichte, d. Deutsch. Chem. Ges. 1906, 39, 4072, and

" " " " " 1907, 40, 1027

the experiments of Ehrlich with saké yeast<sup>2</sup> under different conditions with the view of reducing the formation of fusel oil to the minimum, and of finally applying the method on a large scale for practical purposes.

# I. Experiment.

- (1) 200 g. sugar were dissolved in 2 liters distilled water and put in a capacious flask stoppered with cotton plugs. After repeated sterilizations, the solution was inoculated with 4 g. saké yeast and left for fermentation at the ordinary temperature (20-30°) for 20 days. When the fermentation was finished, the liquid was filtered and analysed. The results was as follows—

Alcohol .....	3.62%
Fusel oil .....	trace.
Total acids (calculated as succinic acid)...	0.0084
Non-volatile acids .....	0.0024
Volatile acid (as acetic acid).....	0.0076
Sugar .....	present (by Molish reaction)
Ammonia .....	absent.

- (2) 200 g. sugar and 4 g. leucine were dissolved in 2 liters water, sterilized and inoculated with 4 g. yeast. The treatment was exactly the same as in experiment (1). After 25 days, when the fermentation was finished the liquid was filtered and analyzed.

Alcohol .....	5.27%
Fusel oil .....	0.125%
Total acids .....	.0084%

2. The sake yeast used in this experiment was a pure culture of (A) No. 25 in kôji-extract. The sediment of the yeast was collected on a sterilized filter, and after washing several times with sterilized water was pressed between the sterilized filter and immediately added to the solutions to be tested. The fresh pressed yeast prepared in this way contained 29.96% dry matter of which 11.90% was nitrogen. Sugar was also previously tested for ammonia and nitric acid.

Non-volatile acids .....	0.0051%
Volatile acids .....	0.0041%
Sugar .....	present.
Ammonia .....	absent.

- (3) 100 g. sugar and 2.15 g. hydrochlorate of glutaminic acid were dissolved in 2 liters water, neutralized with caustic soda, sterilized and inoculated with 29 yeast, kept for 20 days and analysed.

Alcohol .....	5.87%
Fusel oil .....	trace.
Total acid .....	0.0164%
Non-volatile acid .....	0.0124%
Volatile acid .....	0.0051%
Sugar .....	absent.
Ammonia .....	absent.

- (4) 200 g. sugar and 4 g. aspartic acid, were dissolved in 2 liters water, neutralized with caustic soda, sterilized and inoculated with 4 g. yeast, kept for 20 days and analysed.

Alcohol .....	6.39%
Fusel oil .....	0.061%
Total acids .....	0.0128%
Non-volatile acids .....	0.0069%
Volatile acids .....	0.0075%
Sugar .....	absent.
Ammonia .....	present in a small quantity.

- (5) 100 g. sugar and 2 g. glycocoll were dissolved in 1 litre water, inoculated with 2 g. yeast and kept for 20 days.

Alcohol .....	5.33%
Fusel oil .....	0.136%
Total acids .....	0.0164%

Non-volatile acids .....	0.0062%
Volatile acids .....	0.0130%
Sugar .....	present in a small quantity.
Ammonia .....	trace.

- (6) 100 g. sugar and 1.7 g. of the decomposition products<sup>1</sup> of rice protein were dissolved in 1 liter water, inoculated with 2 g. yeast and kept for 20 days.

Alcohol .....	5.28%
Fusel oil .....	0.028%
Total acids .....	0.0221%
Non-volatile acids .....	0.0098%
Volatile acids .....	0.0157%
Sugar .....	absent.
Ammonia .....	present in a small quantity.

- (7) 100 g. sugar and 0.75 g. tyrosine were dissolved in 1 liter water, inoculated with 2 g. yeast and kept for 20 days.

Alcohol .....	3.16%
Fusel oil .....	trace.
Total acids .....	0.0073%
Non-volatile acids .....	0.0048%
Volatile acids .....	0.0032%
Sugar .....	much.
Ammonia .....	trace.

- (8) 50 g. sugar, 1 g. leucine and 1 g. ammonium carbonate were dissolved in 500 c.c. water, inoculated with 1 g. yeast and kept for 18 days.

1. These decomposition products, were obtained by boiling 100 g rice protein, prepared with Ritthausen's method, in 300 c.c. of 25% sulphuric acid for 40 hours. After cooling, the liquid was diluted with water and the sulphuric acid still remaining was carefully removed with baryta, filtered and evaporated in vacuum nearly to dryness, and then dried over sulphuric acid.



Alcohol .....	6.44%
Fusel oil .....	0.153%
Total acids .....	trace.
Non-volatile acids .....	trace.
Volatile acids .....	trace.
Sugar .....	absent.
Ammonia .....	present.

- (9) 50 g. sugar 1 g. leucine and 1 g. ammonium phosphate were dissolved in 500 c.c. water, inoculated with 1 g. yeast and kept for 18 days.

Alcohol .....	6.44%
Fusel oil .....	trace.
Total acids .....	0.0048%
Non-volatile acids .....	0.0040%
Volatile acids .....	0.0011%
Sugar .....	absent.
Ammonia .....	present.

- (10) 50 g. sugar, 1 g. leucine and 1 g. ammonium citrate were dissolved in 500 c.c. water, inoculated with 1 g. yeast and kept for 18 days.

Alcohol .....	7.08%
Fusel oil .....	0.194%
Total acids .....	0.0097%
Non-volatile acids .....	trace.
Volatile acids .....	0.124%
Sugar .....	absent.
Ammonia .....	present.

- (11) 50 g. sugar, 1 g. leucine and 1 g. ammonium tartarate were dissolved in 500 c.c. water, inoculated with 1 g. yeast and left for fermentation for 18 days.

Alcohol .....	5.15%
Fusel oil .....	0.126%
Total acids .....	0.0011%
Non-volatile acids .....	0.0062%
Volatile acids .....	0.0062%
Sugar .....	absent.
Ammonia .....	present.

For the sake of clearness the above results may be presented in a tabular view, as follows:

No.	N-compounds	alcohol %	fusel oil %	total acids %	volatile acids %	non-volatile acids %
1	.....	3.62	trace	0.0084	0.0076	0.0024
2	leucine	5.27	0.125	0.0084	0.0041	0.0051
3	glutaminic acid	5.87	trace	0.0164	0.0051	0.0124
4	aspartic acid	6.39	0.061	0.0128	0.0075	0.0069
5	glycocoll	5.33	0.136	0.0164	0.0130	0.0062
6	decomposition products of rice protein.	5.28	0.028	0.0221	0.0157	0.0098
7	tyrosine	3.46	trace	0.0073	0.0032	0.0043
8	leucine + ammonium carbonate	6.44	0.053	trace	trace	trace
9	leucine + ammonium phosphate	6.44	trace	0.0043	0.0011	0.0004
10	leucine + ammonium citrate	7.03	0.194	0.0097	0.0124	trace
11	leucine + ammonium tartarate	5.15	0.126	0.0011	0.0062	0.0062

We see from the above results, that either in a pure sugar solution (1) or in a solution containing only tyrosine (7) or glutaminic acid (3), doubtful traces of fusel oil were produced; while in a solution containing leucine (2) 0.125% of it was found. This evidently proves the correctness of Ehrlich's view. An exception was found in the glycocoll containing solution (5), which was found to contain 0.136% of fusel oil with Rose's

method. *But this apparent exception was afterwards found to be due to a defect of RÖSE's method.* The substance which behaves same as fusel oil to chloroform was proved to be ethyl-acetate. The latter is easily distinguished from fusel oil by its giving a rose red colouration with anisaldehyde and concentrated sulphuric acid, while fusel oil gives a violet colouration. To make the proof more complete, the distillate of the culture of the glycoecoll solution containing ethyl-acetate was saponified by boiling with dilute caustic kali in a reverted cooler for 4 hours, and was distilled after acidifying with sulphuric acid. The distillate was neutralized with baryta and evaporated. The barium salts were proved to be acetates mixed with a little butyrate. Dr. SHELL<sup>1</sup> has shown that many substances, such as, ethylacetate, amylacetate, aldehydes, furfural and some higher alcohols are absorbed by chloroform and thus bring about the inaccuracy of ROSE's method. In the glycoecoll solution (5) I have tried to find out methyl-alcohol, aldehyde and furfural, but the results were negative.

Ammonium carbonate (8) and phosphate (9), when added to the leucine solution caused more or less diminution in the formation of fusel oil, while ammonium tartarate (11) and citrate (10) had apparently no such effect. As this deserves further consideration further experiments were carried out more fully.

## II. Experiment.

In the following experiments, the formation of fusel oil from leucine under different conditions was especially kept in view.

- (1) 50 g. sugar and 2 g. leucine were dissolved in 500 c.c. distilled water, inoculated with 6 g. yeast and kept for 5 days at 30° C.
- (2) 50 g. sugar without addition of leucine, were dissolved in 500 c.c. water, inoculated with 6 g. yeast and kept for 5 days at 30° C.

1. Dr. E. Sell: über Brantwein (Berlin, 1888) S. 35.

The fermented fluids were analysed with the following results:—

No.	Leucine added	Alcohol	Fusel oil
1	2 g.	6.97 %	0.057 %
2	0	4.67	trace

The total quantity of fusel oil contained in solution 1) was calculated to be 0.287 g. corresponding to 0.292 g. leucine, that is only 15% of the leucine originally added. In the fermental liquid 0.0755 g. N was still found to be present in the filtrate of phosphotungstic acid-precipitate, which includes the whole protein substances together with organic bases. If we suppose that this nitrogen belongs entirely to leucine, then it corresponds to 0.706 g. We may, therefore, conclude that nearly one half of leucine has been split up into other compounds or used up for the formation of yeast cells.

### III. Experiment.

500 c.c. well water, 10 c.c. Hayduck's mineral solution and 50 g. sugar, each were put into 4 Pasteur's flasks and further to flask (1) 2 g. Leucine, to (2) 1.007 g. ammonium phosphate (Equivalent to 2 g. leucine), to (3) 0.733 g. ammonium carbonate and to (4) 1.145 g. glycecoll were added. They were sterilized and inoculated with a minute quantity of saké yeast and kept at the ordinary temperature (20-30°) for 2 months until the fermentation was complete and much sediment of yeast was formed at the bottom. The liquid was then filtered and analysed, with the following result:—

No.		Fusel oil % (Röse's method)
1	leucine	0.279
2	ammonium phosphate	0.138
3	" carbonate	0.098
4	glycocoll	0.242 <sup>1</sup>

## VI. Experiments to prevent the formation of fusel oil.

Since there is no doubt from the above experiments, that fusel oil is chiefly derived from leucine during the fermentation process and further its production is more or less diminished by the addition of ammonium salt to the fermenting liquid, I proceeded one step further to determine the conditions, necessary to reduce it to the minimum. This is especially important from the practical point of view, because saké usually contains a more fusel oil than beer or wine. For example, the samples of superior qualities examined by myself contained about 0.05-0.1% and in those of inferior qualities sometimes as much as 0.4% of it. This higher percentage of fusel oil in saké is most probably due to the comparative richness of the molecules of rice protein in leucine, as shown by U.<sup>1</sup> Suzuki and others of this laboratory.

- (1) 100 g. sugar, 1 g. leucine and 20 c.c. Hayduck's mineral solution were dissolved in 1 liter of water. The solution thus prepared was equally divided and placed in 10 flasks, and to each

1. In this case the apparent increase of fusel oil in the glycocoll solution (4) was due to the formation of ethylacetate. It was at once recognized by its characteristic agreeable smell and by the rose red colouration with anisaldehyde and concentrated sulphuric acid. So I am sure that the decomposition products of glycocoll by sake yeast are chiefly acetic acid and acetic ester.

2. U. Suzuki and others— this Journal; Vol. I., No. I.

flask was added ammonium phosphate in varying quantities. After sterilizing and inoculating with saké yeast, they were left for fermentation at 25° for 2 weeks, when the liquid was filtered and analysed.

No	leucine added	amm. phosphate %	Yeast	Amylalcohol <sup>1</sup>
1	0.1 %	0	not added	—
2	"	0	added	+++
3	"	0.05	"	++
4	"	0.1	"	+
5	"	0.2	"	±
6	"	0.3	"	±
7	"	0.4	"	+
8	"	0.5	"	++
9	"	0.6	"	+++
10	"	0.7	"	+++

We see from this result that fusel oil gradually decreases with the increased addition of ammonium phosphate to the solution and reaches its minimum when the latter becomes 2-3 times of leucine, and again gradually increases with the increase of the latter.

(2) The same experiment was repeated.

1. Amyl-alcohol was tested colourimetrically with anisaldehyde and concentrated sulphuric acid.

— means no reaction, ± trace, + presence of amyralcohol.

No.	leucine added %	amm. phosphate %	Yeast	amylalcohol
1	0.1	0	no added	—
2	"	0	added	+
3	"	0.05	"	+
4	"	0.1	"	±
5	"	0.2	"	±
6	"	0.3	"	±
7	"	0.4	"	±
8	"	0.5	"	+
9	"	0.7	"	+
10	"	0.9	"	+
11	"	1.5	"	+
12	"	2.0	"	0 <sup>1</sup>
13	"	3.0	"	0
14	"	0.1	"	—
15	"	0.2	"	—
16	"	0.5	"	+
17	"	1.5	"	+

In No. 12 and 13 the growth of yeast was absolutely inhibited.

We see in this case also that fusel oil is reduced to the minimum when 2-3 times the ammonium phosphate of leucine were added. We see also that with ammonium phosphate alone, without leucine, a moderate quantity of fusel oil is produced. But if this salt reaches as high as 2% of the liquid then the growth of the yeast comes to a still-stand.

### Summary.

1. Fusel oil is formed in saké chiefly from leucine, which is a decomposition product of rice protein.

2. The formation of fusel oil is diminished in some degree by the by the addition of ammonium carbonate or ammonium phosphate to the fermenting liquid. The best proportion between these ammonium salts and leucine was found to be 2-3:1. The excess of ammonium phosphate not only increases the fusel oil but also prevents the *propagation of yeast cells*. . . Both ammonium citrate and tartarate are useless for this purpose.
3. Glycocoll<sup>1</sup> seems to favour the formation of acetic acid and acetic ester in the fermenting liquid.

In conclusion, I wish to express my thanks to Prof. Dr. U. SUZUKI and Prof. Dr. TAKAHASHI for the valuable suggestions and advices received during the progress of my work.

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1. J. Effront also reported that acetic acid is produced from glycocoll and betain by fermentation (*Zeitsch f. Spirit. indus.* 1909, 32, 237).



## On the Asparagine-splitting Enzyme in Yeast.

BY

K. KURONO.

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The question, whether the formation of fusel oil from leucine is due to the action of a certain enzyme, is not yet settled. Although EHRLICH<sup>1</sup> supposed the existence of such an enzyme, he could not prove it and neither in the "PRESSAFT"<sup>2</sup> nor in the "ACETONE Dauer hefe"<sup>3</sup>, has its presence been shown yet. J. EFFRONT<sup>4</sup> has recently found an enzyme which liberates ammonia from asparagine, and he is inclined to attribute to this enzyme the formation of fusel oil. But as he did not isolate this enzyme in a pure state and further, as it acts only in alkaline solution, while the ordinary fermentation process takes place in a slightly acid fluid, his assumption can not be accepted without further investigation. The isolation of this enzyme from beer yeast as well as from saké yeast, and a fuller examination of its behavior are the subjects of this paper.

### Experiment I.

500 g. well washed bottom yeast were distributed in 1 liter distilled water, and 50 c.c. normal caustic soda was added to it. To prevent bacterial growth the mixture was covered with enough toluol. After keeping for 2 days in a thermostat at 37°, it was filtered. The clear filtrate

1. Berichte, d. Deutsch. Chem. Ges. 1907, Bd. 40, S. 1027.

" " " " " 1906, Bd. 39, S. 4072.

2. E. Buchner u. J. Meissenheimer; Berichte, d. Deutsch. Chem. Ges. 1906, Bd. 39, S. 3713

3. Haus Pringsheim; Berichte, d. Deutsch. Chem. Ges. 1906, Bd. 39, S. 3713.

4. Comptes Rendus, 1908, CXLVI, 779.

was then mixed with an equal volume of 97% alcohol, and the flocky precipitate obtained was collected on a filter and washed with dilute alcohol and ether. About 4 g. crude enzyme was thus obtained, and the following experiments were carried out with it.

- (a) 100 c.c. of 2% asparagine solution was put in a flask of 200 c.c. capacity. 1 c.c. of normal caustic soda and 2 g. (crude enzyme) were added to it. The flask was kept in a thermostat at 27° C, with the addition of enough toluol to prevent putrefaction. At the outset of the experiment a portion was taken out for ammonia determination<sup>1</sup>, while the remaining portions were taken out after 28 and 72 hours respectively.
- (b) For control, 2 g. ppt were dissolved in 100 c.c. water without addition of asparagine, and treated in exactly the same way as before.

Time	NH <sub>3</sub> —nitrogen. %	
	(a) Asparagine solution	(b) Control solution
At the outset ... ..	0.000	6.000
After 28 hours ... ..	0.056	—
After 72 hours ... ..	0.090	0.001

A great difference is thus seen between the control and the asparagine solution as to the production of ammonia. NESSLER's test also reveals this difference at once.

### Experiment II.

300 g. beer yeast were rubbed with some quartz sand in an iron mor-

1. The method used was a qualified vacuum distillation method with finely powdered magnesia usta.

tar, and macerated with 1 liter distilled water. After 2 hours it was filtered. The white precipitate obtained by the addition of 97% alcohol was collected on a filter, and washed with absolute alcohol and ether. About 5 g. air dry crude enzyme were thus obtained. It was divided into 6 parts and the following experiments were carried out.

- (A) 2 g. asparagine, 0.8 g. crude enzyme and 1.5 c.c. normal caustic soda were added to 150 c.c. distilled water and covered with toluol. After keeping in a thermostat at 37° C, ammonia test was made with the following results.

Time .....	$\text{NH}_3\text{—N}\%$
At the outset .....	0.000
After 92 hs. ....	0.112

- (B) The same solution as (A) was boiled for 5 minutes, the treatment being otherwise the same.

Time .....	$\text{NH}_3\text{—N}\%$
At the outset.....	trace.
After 92 hs. ....	0.011

- (C) 2 g. asparagine, 0.8 g. crude enzyme were dissolved in 150 c.c. of water, and further as much tartaric acid was added as was necessary to make it 0.1% of the solution, the treatment being otherwise the same as before.

Time .....	$\text{NH}_3\text{—N}\%$
At the outset.....	0.000
After 92 hs. ....	0.117

- (D) 0.5 g. leucine, 0.8 g. crude enzyme and 1.5 c.c. normal caustic soda were added to 150 c.c. water and treated exactly as before.

Time .....	$\text{NH}_3\text{—N}\%$
At the outset.....	0.000
After 92 hs. ....	trace.

- (E) In this case the caustic soda of the previous solution (D) was replaced with tartaric acid (0.1% of the solution).

Time .....	$\text{NH}_3\text{—N}\%$
At the outset.....	0.000
After 92 hs. ....	trace.
(F) 1 g. urea, 0.8 g. crude enzyme and 1.5 c.c. normal caustic soda were added in 150 c.c. water.	
Time .....	$\text{NH}_3\text{—N}\%$
At the outset.....	0.000
After 92 hs. ....	trace.

The above experiments prove beyond doubt, that an enzyme which decomposes asparagine with the liberation of ammonia. can be easily isolated, and that it acts not only in alkaline, but also and even more energetically in acid solutions. Further its action is confined to asparagine, but leucine and urea being never affected by it.

The writer repeated the same experiments with saké yeast and obtained the same results.

### Experiment III.

30 gr. of saké yeast were rubbed with quartz sand in a mortar, macerated with 300 c.c. of distilled water and filtered. The filtrate was diluted to 300 c.c. and divided into six equal portions, each of which was put in a small flask of ca. 100 c.c. capacity. They were subjected to the same conditions as in the foregoing experiments.

The special additions consisted in:

- (A) 0.5 gr. asparagine.
- (B) 0.5 gr. asparagine after boiling.
- (C) 0.5 gr. asparagine, acidulated with tartaric acid.
- (D) 0.2 gr. leucine.
- (E) 0.2 gr. leucine, acidulated with tartaric acid,
- (F) 0.5 gr. urea.

The determination of ammonia gave the following results:

Series	Ammoniacal N, $\frac{1}{2}$		Nessler's reaction
	at the outset.	after 48hs.	
A	0.070	0.114	very strong reaction with reddish brown precipitates.
B	trace	* 0.059	strong reaction.
C	0.000	0.112	very strong reaction with reddish brown precipitate.
D	0.000	0.000	no reaction
E	0.000	0.000	no reaction
F	0.000	0.000	no reaction

Thus the presence of an asparagine-splitting enzyme in saké yeast is proved.

#### Experiment IV.

300 gr. of beer yeast were rubbed, macerated and filtered as in experiment II. From this filtrate about 5 gr. of crude enzyme were obtained. It was again dissolved in 300 c.c. water, filtered and diluted to 500 and then divided into five equal portions, each of which was put in a small flask of ca. 100 c.c. capacity. They were placed in the same conditions, as in the foregoing experiments.

The special addition consisted in:

- (A) 1 gr. asparagine,
- (B) 1 gr. urea,
- (C) 0.5 gr. leucine.

\* This exception is perhaps due to the too short boiling, in consequence of which the enzyme was not yet completely destroyed.

(D) 1 gr. formamide,

(E) 1 gr. butylamide.

The determination of ammonia gave the following results:

Series	Ammoniacal N, %		Nessler's reaction
	at the outset	after 48 hs.	
A	0.000	0.129	very strong reaction with reddish brown precipitate.
B	0.000	0.000	no reaction.
C	0.000	0.000	no reaction.
D	0.000	trace	weak reaction.
E	0.000	0.000	no reaction.

Thus, it is proved that the action of this enzyme is confined to asparagine.

#### Conclusion.

The presence of an enzyme which liberates ammonia from asparagine is proved in saké as well as in beer yeast. This enzyme can be extracted with water or with a dilute alkaline solution and acts equally well both in acid and alkaline reactions.

As its action is confined to asparagine and is totally inefficient towards leucine, urea etc., it has probably nothing to do with the formation of fusel oil during alcoholic fermentation.

# Studies on the Butyric Acid forming Bacillus of "Saké-Moromi."

BY

K. Kurono.

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With Plates XIII and XIV.

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Although many investigations have been carried on on the microorganisms of "Moromi," the rôle of the butyric acid bacillus still remains to be made out. Moreover, there is the notable fact that the characteristic agreeable odour of butyric ester is usually recognized in the "Takaawa"—"Hochkräusen" stage. These considerations have induced me to undertake the present study.

The samples,<sup>1</sup> which were taken from various stages of "Moromi" fermentation, were boiled at 100°C for 15 minutes in the culture medium,<sup>2</sup> and sealed up in Buchner's tubes for anaërobic culture. They were kept for three days in an incubator at 50°C.

From these cultures,<sup>3</sup> three varieties of butyric acid bacilli have been obtained and are described below.

## No. I.—*Bacillus butyricus aromafaciensis moromi* I.

The principal characteristic of this bacillus is that it produces an agreeable odour of butyric ester in cultures containing alcohol. We can distinguish two varieties:

### I. Form and Size.—Facultative anaërobe; commonly slender rods

1. The samples were taken from a "Sakura-masamune" brewery in 1908 and 1909.
2. As culture media for the isolation of bacteria was used bouillon-glucose-agar with the addition of enough precipitated calcium carbonate.
3. A thin greyish-white film was usually formed in the cultures made from samples taken before the "Takaawa," but not in those taken later.

with slightly rounded extremities; 2 to 4  $\mu$  long by 0.8 to 1.2  $\mu$  thick; in old cultures (moromi-glucose agar after 5 months) long oval, 2  $\mu$  long by 1  $\mu$  thick. Involution forms have never been observed, even in cultures over 4 months old.

II. Flagella and mobility.—Many flagella; actively mobile.

III. Staining reaction.—Stains with Gram's method, but not with methylen-blue or I + KI—solution. Granules found neither in aerobic or anaërobic cultures (starch-bouillon-agar, glucose-bouillon-agar etc.)

IV.—Growth: 1. Solid culture:

- a. Plate culture. — Bouillon-glucose-agar, bouillon-starch-agar, koji-agar: flat, round, light greyish, waxy, slimy colonies of 2-3 m.m. diameter, appear on surface after 4 days at 30°C. Internally the colonies present a finely granular appearance and the periphery is perfectly even when seen under a magnification of 120 diameters.

Frequently large colonies of even  $\frac{1}{2}$  inch diameter are formed. They always present a wavy periphery and seem to spread out along the wet surface. Such a colony obtained after 3 days at 40°C has a thin, dry, somewhat crumpled surface.

- b. Surface culture: "Saké"-agar: Forms a thin, dirty greyish white covering with dry folded surface and quickly growing out on either side of the track with wavy margin. The condensed water becomes turbid and covered with greyish white thick folded film (after 3 days at 25° C). Bouillon-glucose-agar: Growth just the same as on saké-agar, but is covered sooner all over the surface (3 days at 25° C).

Koji-agar: Growth the same as on bouillon-glucose-agar, but the condensed water remain clear (3 days at 25° C).

"Moromi"-agar: Growth quite the same as on bouillon-glucose-agar (3 days at 25° C).

Potato-culture: Forms a dry, greyish white, thick folded



covering and spreads quickly all over the surface (3 days at 25° C).

- c. Stab culture: Koji-agar: Forms a greyish brown wrinkled colony spreading quickly over the surface, and forms thready growth with twisted margin along the stab canal (3 days at 25° C). Bouillon-starch-agar: Cultures similar in character to the preceding.

Bouillon-glucose-gelatine: Forms a greyish white thready growth with twisted margin, diminishing towards the bottoms of the stab canal. First stage thin greyish white smooth pellicle produced at the mouth of the stab canal with dish shaped depression; later stage, the depressed part becomes liquefied and shaped like a crater, the liquid being turbid; final stage, the liquefaction proceeds downwards in the form of a cylinder<sup>4</sup>, with greyish brown wrinkled pellicle on the surface of the liquid (at 15—20° C).

2. Fluid culture: (4 days at 35 C°) Neutral "koji" extract: Forms a greyish white large wrinkled pellicle, the fluid remaining clear; sediment formed; the pellicle is easily scattered in the form of clouds by shaking, but the ring remains unaltered.

Glucose-bouillon: Forms a thick, substantial folded film with some deposits, fluid turbid; the film not scattered by shaking.

Starch-bouillon: Forms a white, somewhat shiny film with some deposits, fluid turbid; the film scatters on shaking.

Yeast-water: Forms a white folded film. Fluid clear. No sediment.

Yeast-water glucose: Forms a white folded film. Fluid clear. No sediment.

4. After 10 days the liquefaction proceeded half way down the thickness of the medium, the liquefying process being most energetic of the three varieties dealt with in this paper.

Hayduck's solution: Forms a thin brittle film with some deposits.  
fluid turbid; in a short time the film sinks and breaks up without leaving any ring.

Mayer's solution: Just the same as above, with the only difference that the ring remains.

Pfeffer's solution: No growth.

Nägeli's solution: No growth.

Milk: Is not coagulated. The growth is very difficult to observe, as no film is formed.

V. Behavior towards carbohydrates, glycerine and calcium lactate. The production of acid from these substances was tested with yeast water cultures containing one of these substances<sup>5</sup>. After 10 days at 35° C, volatile acid was determined by the steam distillation method.

Substance	Volatile acid production
Glucose ... ..	+++- ... 0.15%
Inulin ... ..	++++
Saccharose ... ..	+++
Starch ... ..	+++++ ... 0.2%
Rhamnose ... ..	++
Maltose ... ..	++
Mannose ... ..	/
Mannit ... ..	+
Methyl glucoside ... ..	-
Fructose ... ..	-
Lactose ... ..	-
Melitose ... ..	++
Glycerine ... ..	-
Calciumlactate ... ..	-

Thus this bacillus forms quite a quantity of volatile acid from starch glucose and inulin, and traces of it from rhamnose, maltose and melitose.

The growth is almost the same in all these fluids i.e. a greyish folded film is formed, accompanied by sediment and the fluid becoming turbid.

5. A 2% solution of each substance was used.

VI. Behavior towards nitrogenous compounds: This was observed with various fluids prepared by adding to Hayduck's solution various nitrogenous compounds in place of the asparagin. Tests for aspartic acid, asparagin, leucin, arginin, glutamic acid, cystin, glyceoll, tyrosin, histidin, alanin, ammonium carbonate and potassium nitrate, showed that if due attention was paid for acidity, nitrogen was assimilable in amino-ammonia- and nitrate forms.

VII. Behavior towards acids: This bacillus can grow in "koji"-extracts having an acidity less than 0.089% as lactic acid, but not in 0.13%; but, when we add pure lactic acid to the neutral "koji"-extract, it can grow when the acidity is less than 0.05%.

VIII. Behavior towards antiseptics. Only salicylic acid was tested. 0.01% of it in "koji"-extract inhibits the growth completely, but 0.004% does not.

IX. Behavior towards alcohol. Less than 6.25 vol.% of ethyl-alcohol in "koji"-extract does not inhibit the growth. Further, the agreeable odour of ethyl-ester is naturally noticeable in cultures of this bacillus when ethyl alcohol is present.

X. Although aroma-formation was tested with various alcohols<sup>6</sup>, only ethyl alcohol gave a positive result.

XI. Fermentation products. Traces of aldehyde, ammonia, ethyl alcohol and fusel-oil are found in the distillate of "koji"-extract-culture after 10 days at 25° C, but methyl-alcohol, furfural, acetone, acrolein indol and hydrogen sulphide are not present. Butyric acid is an essential component of volatile acids, and a trace of acetic acid is found but formic acid and propionic acid are not present.

XII. Gas formation. No formation of gas.

6. Ethyl-alcohol, methyl-alcohol, normalpropyl-alcohol, isopropyl-alcohol, normalbutyl alcohol, isobutyl-alcohol, cinnamyl-alcohol, amyl-alcohol, heptylic-alcohol, cuminic alcohol, and caprylic alcohol were tested by adding traces of them to Hayduck's solution.

XIII. Conditions of temperature: Optimum temperature for growth lies 30—40° C. Grows very slightly below 13°C. Boiling for 2 hours in distilled water does not kill the spores of this bacillus, which, however, are completely killed by boiling for 3 hours.

XIV. Spore formation: Long oval spores are formed after 2 days at 40° C. medially situated. Starch-bouillon and "koji"-agar were most favourable for spore formation.

XV. Symbiotic culture of "saké"-yeast and this bacillus: These 2 microorganisms were added to "koji"-extract and kept at 30° C; after 2 days enough growth of the bacillus took place to produce the odour of butyric acid, but the yeast grew slightly; after 4 more days the yeast grew tolerably well, but the emission of the agreeable odour of butyric ester was replaced by the bad smell of butyric acid.

#### No. 2. *Bacillus butyricus aromafaciensis moromi* II.

This variety differs from the first in that it does not grow in saké-agar. It however grows in Nägeli's solution, while the first variety does not.

I. Form and size: Almost the same as in the first variety.

II. Flagella: Same as in the first variety.

III. Staining reaction: Same as in the first variety.

IV. Growth: 1. Solid culture:

a. Plate culture: Almost the same as in the first variety.

b. Surface culture: bouillon-glucose-agar, "koji"-agar, "Moromi"-agar: Grows just the same as the first variety. "Saké"-agar: No growth. Potato culture: Forms a folded covering, which is whiter than in the first variety.

c. Stab culture: Koji-agar, bouillon-starch-agar: Almost the same as in the first variety. Bouillon-glucose-gelatine: Liquefaction proceeds more slowly than in the first variety (about half as first).

2. Fluid culture: (4 days at 35° C). Neutral "koji"-extract: Forms a greyish white wrinkled pellicle, fluid becoming

turbid with sediment; this pellicle is easily scattered like clouds on shaking and no ring remains.

Neutral "Moromi": Same as above. Bouillon-glucose, starch-bouillon, yeast-water, and yeast-water-glucose: Grows respectively as in the first variety.

Hayduck's solution: Forms a thick, folded, slimy, semi-transparent film, no deposits, medium slightly turbid; on shaking, this film does not scatter but sinks down.

Mayer's solution: Growth same as in the first variety.

Pfeffer's solution: No growth.

Nägeli's solution: Forms no film; medium slightly turbid with some white sediment; scatters like powder on shaking.

Milk: Growth same as in the first variety.

V. Behaviour towards carbohydrates, glycerine and calcium lactate: The production of volatile acid was determined under the same conditions as in the first variety with the following results.

Substance	Volatile acid production	
	First variety	Second variety
Glucose ... ..	++++	+++
Inulin ... ..	++++	+
Saccharose... ..	+++	+++
Starch ... ..	+++++	++++
Rhamnose ... ..	++	++
Mannose ... ..	/	—
Maltose ... ..	++	+
Mannit ... ..	+	+
Methyl glucoside ... ..	±	+
Fructose ... ..	—	—
Lactose ... ..	—	—
Melitose ... ..	++	++
Glycerine ... ..	—	—
Calcium-lactate ... ..	—	—

On the whole, the acid producing power of this bacillus is less than in the first variety. Although the largest amount of acid was produced in the cases of starch and glucose, it amounted to only 0.128 and 0.113% respectively, as butyric acid. Moreover, the acid production from inulin is far less than in the first variety.

VI. Behavior towards nitrogenous compounds. The results were analogous to those obtained in the first variety.

VII. Behavior towards acids and alcohol; aroma-formation, gas-formation, fermentation products, conditions of temperature, spore formation and symbiotic culture with saké-yeast gave the same results as in the first variety.

VIII. Behavior towards antiseptics: This bacillus is more resistant than the first variety towards salicylic acid, 0.01% of it having no power to inhibit the growth.

Although numerous butyric bacilli have been described, the present form cannot be referred to any of them. The distinguishing properties of this variety are its facultative anaërobiosis, active mobility, and absence of granules and clostridium. Moreover, this bacillus produces butyric acid principally from starch, glucose, etc., but not from milk-sugar or calcium lactate; further it has no power to ferment or coagulate milk. There is no doubt that this bacillus is distinct from *Bac. butyricus* Hüppe, *Bac. Gruber* III, and other butyric bacilli described by L. Löffler, A. Weber, L. Adametz and O. Emmerling, although there are several characteristic common to them and the new form.

In short, this bacillus may be considered a new species of butyric bacillus resembling the other genuine butyric bacilli physiologically (i.e. in producing butyric acid from carbohydrates as starch and glucose) and standing morphologically nearest to *Bac. butyricus* Hüppe, *Bac. butyricus* Weber, etc. Moreover, it is characteristic for this bacillus that it produces the agreeable odour of butyric-ester in the presence of ethyl-alcohol. On

this point *Bac. esterificans* Massen<sup>7</sup> shows an analogous action, but it produces ananas aroma in the butter without alcohol.

### No. 3. *Bacillus butyricus roseus moromi*.

- I. Form and size: Almost the same as in No. 1 and No. 2.
- II. Elagella: Just the same as in No. 1 and No. 2.
- III. Staining reaction: Similar to the foregoing.
- IV. Growth: 1. Solid culture:
  - a. Plate culture: almost the same as in No. 1 and No. 2; but the colonies produced in the deeper parts of the medium is ball shaped, with irregular surface and not round and flat as in No. 1 and No. 2; moreover, such colonies after 3 days at 40° C do not present the dry, slightly crumpled surface of No. 1.
  - b. Surface culture: "Saké"-agar: no growth as in No. 2. Moromi-agar, bouillon-glucose-agar and "koji"-agar: Forms a greyish white, wet lustrous covering. It grows quickly, spreading out on either side of the track and ultimately covering the whole surface, but never presenting a dry folded surface. The condensed water becomes turbid with some white sediment, but no film is formed (3 days at 25° C).

Potato culture: Forms a wet, lustrous, dark red covering<sup>8</sup> spreading quickly over the whole surface (3 days at 25° C).

This is a characteristic of this bacillus differentiating it from No. 1 and No. 2.
  - c. Stab culture: Koji-agar, bouillon-starch-agar: although it grows almost like No. 1 and No. 2, it produces never a dry folded film but a wet, smooth, lustrous one on the surface. Bouillon-glucose-gelatine: growth about the same as in No. 1 and No. 2; but the liquefaction of gelatine proceeds more slowly than in No. 2,

7. H. Huss; Cent. f. Bact.; II. Ab. 569, 1907.

8. A similar red covering is formed also in rice and bread cultures.



that is after 10 days. The dish-shaped depression is found only at the mouth of the stab canal (at 15°-20° C).

2. Fluid culture: (4 days at 35° -C) Neutral "koji"-extract: Forms a white, semi-transparent, smooth pellicle, the fluid remaining clear. Pellicle sinks easily but does not scatter on shaking, and a ring remains.

Neutral "moromi": Same as in koji-extract.

Bouillon-glucose: The medium turns turbid with some cloudy sediment; the pellicle sinks gradually and a ring remains.

Starch-Bouillon: Forms a white smooth pellicle with a white sediment in the turbid fluid; the pellicle scatters like clouds on shaking.

Yeast-water: Forms almost the same growth as in bouillon-glucose culture.

Yeast-water-glucose: Forms almost the same growth as in bouillon-glucose-culture.

Hayduck's solution: Forms a thin slimy film, with some deposits, the fluid becoming slightly turbid; this film does not scatter on shaking

Mayer's solution: Almost the same growth as in Hayduck's solution.

But there is a trace of a ring.

Pfeffer's solution: No growth.

Nägeli's solution: No growth.

Milk: Almost no growth.

V. Behavior towards carbohydrates, glycerine and calcium lactate: The production of volatile acid was determined under the same conditions as in No. 1 and No. 2. The results were as follows:

Substance	Volatile acid production		
	Bacillus No. 1.	Bacillus No. 2.	Bacillus No. 3.
Glucose ... ..	++++	+++	++
Inulin ... ..	++++	+	—
Saccharose ... ..	+++	+++	++
Starch ... ..	+++++	+++	+++
Rhamnose ... ..	++	++	±



Mannose ... ..	/	—	±
Maltose ... ..	++	+	++
Mannit ... ..	+	+	±
Methyl glucoside ... ..	±	+	±
Fructose ... ..	—	—	—
Lactose ... ..	—	—	—
Melitose ... ..	++	++	±
Glycerine ... ..	—	—	—
Calcium lactate ... ..	—	—	—

On the whole, the acid producing power of this bacillus is weaker than in No. 1 or No. 2. Even in the cases of starch and glucose, the acid produced amounted to only 0.094 and 0.082% respectively, as butyric acid. It is a characteristic of this bacillus that it does not form any acid from inulin; and in this it is more like No. 2 than No. 1.

VI. Behavior towards nitrogenous compounds: Results similar as in No. 1 and No. 2.

VII. Behavior towards acid: This bacillus grows in 'koji'-extracts containing less than 0.042% acid as lactic acid, but not when the acidity is more than 0.084%. When however pure lactic acid is added to a neutral "koji"-extract, it grows while the acidity is less than 0.05 but not in 0.06%.

VIII. Behavior towards antiseptics: Just the same as in No. 1.

IX. Behavior towards alcohol: Its power of resistance towards alcohol is so weak that it cannot grow in the presence of 3% alcohol or more. It does not produce butyric ester in the presence of alcohol; and this also distinguishes it from No. 1 and No. 2.

X. The aroma formation from various alcohols and by symbiotic culture with yeast was tested on this bacillus but with negative results.

XI. Fermentation products: Just the same as in No. 1 and No. 2.

XII. Gas formation: No gas.

XIII.—Conditions of temperature: Optimum temperature for growth

is 30-40° C. Grows very slightly below 13° C. Boiling for 1½ hours in distilled water does not kill the spores of this bacillus, which die only when boiled for 2 hours.

XIV. Spore formation: Same as in No. 1 and No. 2.

Although this bacillus closely resembles No. 1 and No. 2, it can be distinguished by its property of not forming an ester from ethyl alcohol and of not causing butyric fermentation in inulin as others do and by its producing a very characteristic dark red covering in potato culture. Comparison with No. 1 and No. 2 leaves no doubt that this is distinct from any of the butyric bacilli already known.

### Summary.

The three new varieties of butyric bacillus found in the "Takaawa" stage of "moromi"-fermentation are,

1. *Bacillus butyricus aromafaciensis moromi* I.
2. *Bacillus butyricus aromafaciensis moromi* II.

3. *Bacillus butyricus roseus moromi*. These 3 bacilli produce butyric acid chiefly from starch and glucose, and 1 and 2 produce the characteristic odour of butyric ester in the presence of ethyl-alcohol or by symbiotic culture with "saké"-yeast; moreover, these 2 bacilli grow in the presence of as much alcohol as ca. 6%. There is no doubt that these 2 bacilli never cause any putrefaction in "saké" and "moromi", because their resisting power towards acids is comparatively very weak. It may therefore be concluded that these 2 bacilli play an important rôle in producing the characteristic aroma of "Takaawa" in "saké" brewing. They may therefore perhaps prove moderately useful for this purpose in future.

*Bacillus* No. 3 on the contrary does not appear to play such an important part, but may be considered as an agent in the production of "akamoto," because it produces a red color in starch cultures.

9. During the saké fermentation, "moto"-mash sometimes changes to a dark red color, when we call it "akamoto."

## Explanation of Figures.

## Plate XIII.

Fig. 1. *Bacillus butyricus aromafaciens moromi* II; after 2 days in "koji"-agar 30°C (1500/1).

Fig. 2. *Bacillus butyricus aromafaciens moromi* I; flagella stained, after 17 hours at 30°C (1500/1).

Fig. 3. *Bacillus butyricus aromafaciens moromi* I; anaerobic culture; 20 hours in "koji"-extract at 30°C (1500/1).

Fig. 4. *Bacillus butyricus aromafaciens moromi* I; spores after double staining; 2 days in "koji"-agar at 40°C (1500/1).

Fig. 5. *Bacillus butyricus aromafaciens moromi* I; 2 days in "koji"-agar at 30°C (1500/1).

Fig. 6. *Bacillus butyricus aromafaciens moromi* II; flagella stained; after 17 hours at 30°C (1500/1).

Fig. 7. *Bacillus butyricus aromafaciens moromi* II; anaerobic culture; after 20 hours in "koji"-extract at 40°C (1500/1).

Fig. 8. *Bacillus butyricus aromafaciens moromi* II; spores after double staining; after 2 days in "koji"-extract at 40°C (1500/1).

Fig. 9. *Bacillus butyricus roseus moromi*; after 2 days in "koji"-agar at 30°C (1500/1).

Fig. 10. *Bacillus butyricus roseus moromi*; flagella stained; after 17 hours at 30°C (1500/1).

Fig. 11. *Bacillus butyricus roseus moromi*; anaerobic culture; after 20 hours in "koji"-extract at 30°C (1500/1).

Fig. 12. *Bacillus butyricus roseus moromi*; spores after double staining; after 2 days in "koji"-extract at 40°C (1500/1).

## Plate XIV.

Fig. 13. *Bacillus butyricus roseus moromi*; plate culture in starch-bouillon-agar after 2 days at 40°C; (a) are deep colonies.

Fig. 14. *Bacillus butyricus roseus moromi*; plate culture in glucose-bouillon-agar after 2 days at 40°C; typical spreading out of a surface colony.

Fig. 15. *Bacillus butyricus aromafaciens moromi* I; plate culture in starch-bouillon-agar, after 2 days at 40°C.

(a) are the colonies formed in a deep position.

Fig. 16. *Bacillus butyricus aromafaciens moromi* II; plate culture in starch-bouillon-agar, after 8 days at 40°C. (a) are colonies formed in a deep position.

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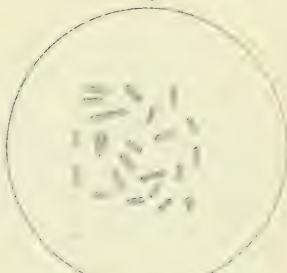
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5.



9.



2.



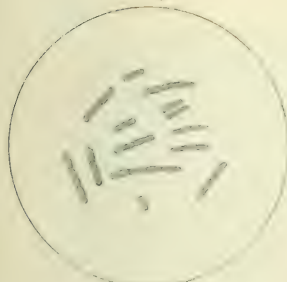
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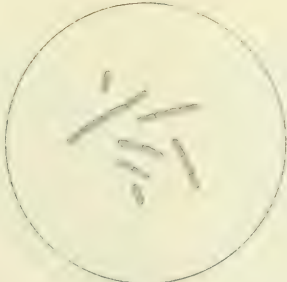
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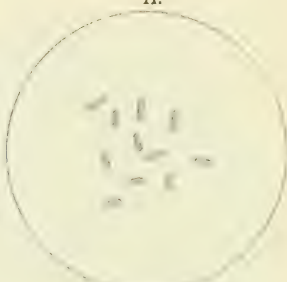
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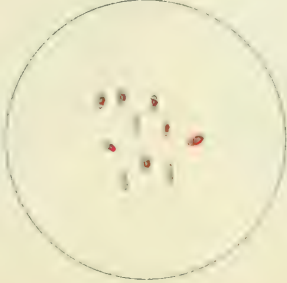
11.



4.



8.



12.





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Fig. 13.

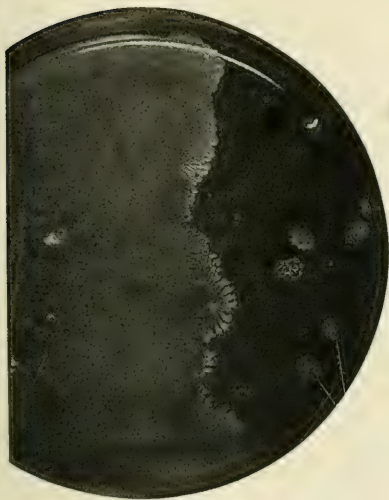


Fig. 14.



Fig. 15.

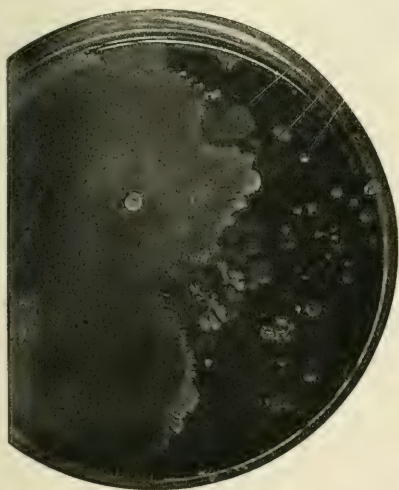
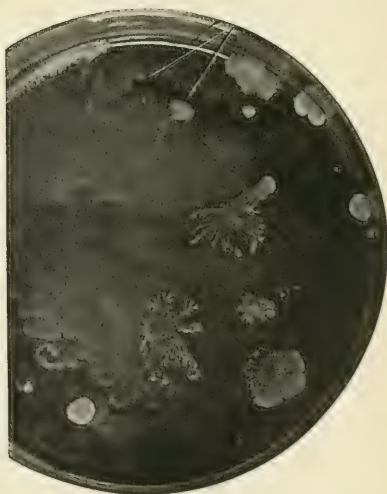


Fig. 16.







## On the Lactic Acid Bacillus of "Moto"-mash.

BY

Y. Okuda.

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Although the lactic acid bacillus of saké has been studied by several authors, there are some points which are still in need of further investigation.

O. Kellner, Y. Mori, and M. Nagaoka<sup>1</sup> found that "Koji"<sup>2</sup> is liable to turn sour by the production of lactic acid, if it is stored in a damp room with insufficient ventilation. T. Okumura<sup>3</sup> found that lactic and acetic fermentations are sometimes observed in the "moto"-mash<sup>4</sup>. It was also pointed out by Y. Kozai<sup>5</sup> that in the earlier stage of "Moto"-preparation lactic acid fermentation takes place, which is favorable for yeast. T. Takahashi<sup>6</sup> has found six species of lactic acid bacilli from altered "Saké" called "Hiyochi-Saké," and one variety has been obtained by K.

1. The Bulletin of the College of Agriculture, Tokyo Imperial University. Vol. I, No. 5, P. 28.

2. "Koji" is steamed rice upon which the mycelium of a particular species of fungus has been developed.

3. Bull. of the Agr. College, Tokyo Imps. University. Vol. IV, P. 212.

4. "Moto"-mash or "Saké moto" is prepared by mixing steamed rice, rice "Koji" and Water.

5. Centralbl. f. Bakteriologie. 1900, II Abt., Bd. VI, S. 392.

6. Bull. of the Agr. College, Tokyo Imp. University. 1907, Vol. VII, 4, 531.

Eda<sup>7</sup> from "moto"- and "Moromi"-mash. Recently S. Mori<sup>8</sup> made a quantitative determination of lactic acid at every stage of "Moto"-preparation. K. Eda<sup>9</sup> added some pure cultures of lactic acid bacillus to the preparation of "Moto." T. Shimoyama and T. Andō<sup>10</sup> arrived at the conclusion that a suitable application of this bacillus simplifies the "Moto"-process. The fact affirms the good effect of the application of lactic acid in the preparation of moto-mash, made by U. Yamagata in 1901.

Cultures of the lactic acid bacillus, *Bacillus Delbrücki*<sup>11</sup> are used in the distillery, but in the case of "Moto"-mash the best kind of bacillus is not known yet, therefore, it has seemed to the writer that an investigation of these micro-organisms of "Moto"-mash will bring out something of importance and interest both from the practical and the scientific point of view.

The samples used for the investigation were obtained from Nada<sup>12</sup> and Fushimi<sup>13</sup> early in January, 1909, and as culture media for the isolation of the microbes, sterilised "Koji"-extract-agar and bouillon-agar with calcium carbonate were used, according to the method of Beyerink<sup>14</sup>. All the microbes isolated by the writer unfortunately produce comparatively small quantities of acid. The breweries which supplied the samples the stages of "Moto"-mash at which they were taken are:—

7. Jyōzōshikenjo Hōkoku (Bulletin of the Imperial Brewing Experiment Station). 1907, 14, 5.
8. " " " " 1909, 25, 68.
9. " " " " 1909, 25, 22.
10. " " " " 1909, 28, 45.
11. Lafar: Handbuch der technischen Mykologie 1906, V, 297.
12. Yamamura's and Ōkura's saké factories at Nada in the province of Settsu.
13. Ōkura's saké factory at Fushimi in the province of Yamashiro.
14. Centralblatt für Bakteriologie. 1891, Abt. I, Bd. IX, S. 781.

Bacteria.	Place.	Stage.
Bac. No. I. ... ..	Yamamura's factory at Nada.	Wakitsuki ("Ankommen").
Coccus No. IV. ... ..	" " " "	"
Bac. No. II. ... ..	Ōkura's " " "	"
Coccus No. I. ... ..	" factory at Fushimi.	Takaawa ("Kräusen")
Coccus No. II. ... ..	" " " "	"

## PART I. BACILLUS.

## Bacillus No. I. (Bac. Aderholdi var moto).

I. Form and size: Rod shaped, 3-5  $\mu$  long, 1  $\mu$  broad, both ends of the cell are round and the two sides are parallel with each other (3 days at 33-35° C in bouillon). Usually, isolated or united in pairs, rarely in chains. In a culture on bouillon-agar somewhat elongated forms were found after two months, but there were no remarkable involution forms. Stains with Gram's method. Non-motile and sporeless.

II. Growth: 1. Solid culture: *a. Plate cultures*:—Bouillon-agar or 10% cane sugar-bouillon-agar: Very small, grayish white colonies appear on the surface of the medium after 3 days at 33-35° C. The periphery of the colony is smooth and the inner part is homogeneous, when seen with a magnification of 170. *b. Stab-cultures*: "Saké"-agar:—A thread like, grayish white growth extends along the whole length of the stab-canal, almost no surface growth (24 hours at 35° C., and 6 days more at the room temperature). Bouillon-gelatine: A grayish white beaded growth along the stab canal. No liquefaction of gelatine after 9 days at the room temperature. *c. Surface culture*: Bouillon-agar; "koji"-extract-agar, 10% cane sugar bouillon-agar: Forms a very feeble, grayish white growth on the surface of the medium, and turbid condensed water with a little sediment after 2 days at 30-33° C. The condensed

water became clear after 17 days. Bouillon-gelatine: Faint growth. No liquefaction after 20 days at the room temperature.

2. Fluid culture: "Koji"-extract at 30-35° C:—This is a very suitable medium for the growth of this bacillus. After one day diffuse turbidity. After two days, increased turbidity, and abundant sediment. When the tube is shaken, the sediment rises in the form of filaments and then immediately scatters like cloud. The fluid remains clear and the sediment appears gray in color (after 25 days). Bouillon: Fluid turbid, but growth not so good as in "koji"-extract. Wort (without hop): Intense turbidity after 3 days at 30-35° C. Yeast-water: Almost no growth after 7 days at 30-33° C. Artificial solution A<sup>15</sup>: No growth even after 25 days at 30-35° C. Artificial solution B<sup>16</sup>: Slight growth.

"Saké"<sup>17</sup>: No growth even after 32 days at 28-33° C. Diluted "Sake" (Alcohol 11.4 weight percent): Fluid turbid, some sediments, after 32 days at 28-23° C. "Moromi"-mash<sup>18</sup>: A slight growth after 30 days at 28-33° C. Beer<sup>19</sup>: No growth after 32 days at 28-33° C. Milk: No coagulation after 22 days at 30-33° C.

15. Asparagin ... ..	1.00 %	Sodium chloride ... ..	0.50 %
Magnesium sulphate... ..	0.02 %	Potassium nitrate ... ..	0.02 %
Potassium monophosphate ...	0.10 %	Glycerin ... ..	1.00 %
Ammonium Carbonate ... ..	0.05 %		

16. Water... ..	1000 c.c.	Calcium chloride ... ..	0.2 gr.
Potassium monophosphate ...	2.0 gr.	Saccharose ... ..	60.0 "
Magnesium sulphate... ..	0.2 "	Peptone ... ..	10.0 "
Sodium carbonate ... ..	1.0 "		

17. Yuwao Tejima analyzed this with the following results:—

Alcohol ... ..	17.24 % (vol)	Glycerin ... ..	0.133 %
Extractive matters ... ..	2.95 "	Ash ... ..	0.049 "
Glucose ... ..	0.017 "	Total acid (as lactic acid) ...	0.297 "
Dextrin ... ..	0.011 "	Volatile acid (as acetic acid)	0.035 "

18. It contained 11.54% of alcohol and 0.409% of total acid as lactic acid.

19. It contained 6.5 % of alcohol and 0.187 % of total acid as lactic acid.

III.—Behavior towards carbohydrates, and comparison with the already described bacilli: the production of acid from carbohydrates was tested with yeast-water containing three per cent of a carbohydrate, during 10 days at 28-33° C. The results are as follows:—

Substance.	Growth <sup>20</sup>	Acid production <sup>21</sup>		
		$\frac{N}{10}$ NaoH.	Bac. No. I.	Bac. Aderholdii.
Rhamnose ... ..	K	0.0	—	—
Glucose ... ..	G	1.6	++	+
Saccharose ... ..	G	2.2	+++	+
Fructose ... ..	G	1.6	++	+
Galactose ... ..	W	0.9	+	+
Maltose ... ..	G	3.8	++++	+
Lactose ... ..	W	0.8	+	+
Raffinose ... ..	W	0.2	(+)	+
Dextrin ... ..	G	1.0	+	+
Inulin ... ..	G	1.2	+	—
Starch ... ..		0.3	(+)	—
Mannit ... ..	K	0.0	—	—
$\alpha$ -methyl-glucoside ... ..	W	0.0	—	—

Growth: Saccharose, fructose, glucose and dextrin: Fluid is almost clear. On shaking, sediment rises, and scatters like cloud. Lactose, galactose,  $\alpha$ -methyl-glucoside: Faint growth. Mannit, rhamnose: No growth. Raffinose: Almost no growth. Inulin: Turns very slightly turbid, and a sandlike sediment is produced at the bottom.

The increased amount of acid was titrated with a decinormal solution of sodium hydroxide, the indicator being phenol-phthalein.

20. In the table, G, indicate good growth; W, slight; K, not.

21. +, indicate production; —, not; (+), trace.

On shaking the sediment diffuses uniformly. Maltose: Fluid is very turbid. (10 days at 28-33° C).

Thus we see that, the growth of this bacillus in the various yeast-waters containing carbohydrates is not so good as that of others described in this report. Among the above mentioned carbohydrates, *maltose* produces, in the interval of time and at the temperature given above, the greatest quantity of acid, but it is far less than in the case of "koji"-extract.

Comparison: We can easily find that this bacillus nearly agrees with *Bacillus Aderholdii* Henneberg<sup>22</sup>, in the production of acid from the above named carbohydrates, *except inulin*, and also in its failure to grow in beer, but the decisive difference between them is that milk is coagulated by the latter and not by the former. It differs from T. Takahashi's<sup>23</sup> *Bacillus Aderholdii* var *Saké* by its action upon inulin and starch, and by the thermal death point. This is also distinguishable from K. Eda's<sup>24</sup> bacillus by its property of production of acid from lactose, inulin, starch, and by many other points.

IV.—Fermentation products: Lactic acid<sup>25</sup>, acetic acid, fusel oil (trace), ammonia, and methyl alcohol were found in the distillate or residue of glucose-yeast-water culture after 21 days at 28-33° C., but formic acid, butyric acid, methyl lactate, ethyl alcohol, aldehyde, acetone, furfural, succinic acid and indol were not found in it.

The increase in the quantity of total acid and non-volatile acid, in 100 c.c. of "koji"-extract after 12 days at 35° C. was titrated with  $\frac{N}{10}$  NaOH. Required c.c. of this alkali solution was as follows:—

Total acid .....	57.0
Non-volatile acid .....	45.5

22. Zeit. f. Spiritus industrie. 1903, 31, 343.

23. Bull. of the Agr. College, Tokyo Imp. University. 1907, VII, 4, 546.

24. Jōzōshikenjo Hōkoku (Bull of the Imp. Brewing Experiment Station). 1907, 14, 13.

25. It was detected with Uffelmann's test and with crystals of Zinc lactate.

V.—Behavior under different temperatures: Optimum temperature for growth lies near 36° C. There is no apparent growth below 17 C., and above 57° C. The test was done with "koji"-extract, and yeast-water-glucose. Generally the growth in "Koji"-extract was much better than in yeast-water-glucose.

Temperature for acid production: Acid is produced very quickly above 30° C. in yeast-water-glucose but its production is more greater in the long run at 26° C. than at 30° C. The minimum and maximum temperatures for acid production are near 17° C. and 52° C. respectively. This test was repeated with "koji"-extract and by titration. According to this result the optimum temperature for the production of acid for long periods, lies near 26° C., and there was an increase of total acid by 0.899% as lactic acid after 30 days.

Thermal death-point: Heating to 70° C. for 10 minutes exceedingly retards the growth, but does not kill all the cells, which die at 80° C. in 10 minutes (in "koji"-extract).

VI.—Behavior towards alcohol, and lactic acid: In "koji"-extract containing 0.119% of total acid (as lactic acid), 3 per cent of alcohol accelerate the growth of this bacteria. With 11% of alcohol it grows well, but with 16% no development is observable after 15 days at 30° C. This bacillus can not grow in yeast water containing 10% of cane sugar beside, 0.47% of lactic acid; while with 0.17% of lactic acid, it grows energetically.

VII.—Relation to the quantity of sugar: For this purpose bouillon containing glucose was used, the culture being continued for 15 days at 26-29° C.

Percentage of glucose	2.	5.	10.	15.	20.	25.	30.	40.	50.
Growth. ... ..	+	+	+	+	+	+	(+)	—	—
	+	+	+	+	+				
	+	+	+	+	+				

VIII.—Necessity of air: Both with Buchner's and Botkin's methods proceeds energetically growth, therefore this bacillus grows well without oxygen.



IX.—Some relation between this bacillus and yeast: Bac. No. I was mixed with saké yeast—viz. B. No. 21 of Jōzōshikenjo,—in the following ratios, in 50 c.c. of “koji”-extract, and after 7, 10, and 27 days at 25-28° C., the number of the cells of the yeast was determined, and the acidity of the medium was titrated with  $\frac{N}{10}$ -NaOH solution.

Remarks. Original medium 10 c.c. corresponds to 1.4 c.c.  $\frac{N}{10}$  NaOH.

Number of yeast cells in 1 c.c. of original culture=7300000  
=7.3 million.

Number of yeast cells in 3 drops of original culture=0.876  
million

1 c.c. pipette used to Bac. No. I.=23 drops.

1 c.c. pipette used to saké yeast=25 drops.

In the following table, to the counting of yeast cell, one million was adopted as the unit.

## A.

Mixed ...	Yeast	3 drops	3 drops	3 drops	3 drops	3 drops	3 drops	3 drops	Zero
	Bacillus	Zero	1 drop	3 drops	0.5 c.c.	0.9 c.c.	2.0 c.c.	1 drop	
After 7 days	Yeast cell in 1 c.c.	162.0	160.0	169.8	151.0	124.8	127.8	0	
„ 27 „	„	169.5	187.0	173.0	—	161.0	151.0	0	
„ 7 „	Acidity <sup>27</sup>	1.7	4.2	4.9	5.7	6.0	6.6	5.6	
„ 27 „	„	1.7	10.7	10.1	—	10.2	10.5	—	

## B.

Mixed ...	Yeast	3 drops	3 drops	3 drops	3 drops	3 drops	3 drops	3 drops	Zero
	Bacillus	Zero	3 drops	5 drops	0.6 c.c.	1.6 c.c.	3.0 c.c.	3 drops	
After 10 days	Yeast cells in 1 c.c.	136.8	171.3	160.3	147.4	150.0	147.8	0	
„ „ „	Acidity	1.8	5.7	6.5	6.6	7.2	7.1	5.6	

27. c.c. of  $\frac{N}{10}$  NaOH required for neutralization of 10 c.c. of the culture medium.



The results are somewhat irregular, but we can see at least, that this yeast grows energetically in spite of the presence of immense numbers of this bacillus, although too many of it are not favorable for the growth of the yeast. Further we see that this bacillus, in mixed cultures, produces acid as much as in pure cultures.

Bacillus No. II. (*Bacillus lactis acidi* Leichmanni var. moto).

I. Form and Size: Rod shaped  $2\frac{1}{2}$ -6  $\mu$  long,  $1\frac{1}{5}$   $\mu$  broad, both ends of the cell are round, and the two sides are parallel with each other. The cells are usually united in chains, and rarely isolated (in bouillon, after 3 days at  $35^{\circ}$  C.). Motile. Stains with Gram's method. Some involution forms were found in a "koji"-extract-agar culture after 40 days.

II. Growth: 1. Solid culture: (a) *Plate cultures*: Bouillon- or saccharose-bouillon-agar: Round or nearly round, yellowish white, somewhat viscous colonies with more or less elevated centre. The periphery is smooth or slightly wavy, and the internal part is homogeneous, when seen with a magnification of 170 (2 days at  $30^{\circ}$  C.). Bouillon-gelatine: Liquefies in the form of hemispheres, and the liquefied part is turbid, and in its central part exist some yellowish deposits (5 days at room temperature). (b) *Stab-cultures*: "Saké"-agar: Forms a faint growth along the stab canal, and a yellowish white, creamy surface growth covering the total surface of the medium (5 days at  $35^{\circ}$  C.). "koji"-extract-agar: Similar as in the case of "Saké"-agar, but the color of the surface growth is dirty gray (4 days at  $35^{\circ}$  C.). Bouillon-gelatine: A funnel-shaped liquefaction occurs, and the liquefied part is turbid (9 days at room temperature). (c) *Surface cultures*: Bouillon-agar or saccharose-bouillon-agar: A yellowish milky growth appears on the needle track. Condensed water is turbid and a yellowish sediment is found at the bottom (2 days at  $35^{\circ}$  C.). After 17 days condensed water is clear. "Saké"-agar: A yellowish white, flat surface growth. Exceedingly turbid con-

densed water with a yellowish white sediment (24 hours at 35° C. and 2 days more at the room temperature). "koji"-extract-gelatine: Liquefaction occurs slowly along the needle track.

2. Fluid culture: koji-extract: Turbid after 24 hours at 35° C. An abundant sediment is found at the bottom. It scatters like cloud on shaking (after 3 days). Islands appear after 5 days and the medium remains clear after 16 days. Bouillon: turbid after 24 hours at 35° C. Becomes clear after 6 days. Yeast-water: Fluid is slightly turbid and sediment somewhat viscous (3 days at 35° C.). Artificial solution A: Becomes turbid, and a small quantity of sediment is found after 10 days at 25-27° C. "Saké", diluted "saké", beer: No growth after 32 hours at 28-33° C. Milk: No change after 3 days at 30° C., after 5 days the upper half of the milk becomes yellowish and transparent, the lower part is being viscous. After 15 days the total medium remains completely clear.

III. Behavior towards carbohydrates and comparison with the already described bacilli: Tests made were the same as in the case of Bac. No. I.

Substance	Growth	Acid production	
		Bacillus lactisacidi Leichmanni.	Bac. No. II
Rhamnose ... ..	G	—	—
Glucose ... ..	G	+	+
Saccharose ... ..	G	+	++
Fructose ... ..	G	+	+
Galactose ... ..	G	+	+
Maltose ... ..	G	+	+
Lactose ... ..	G	+	+
Raffinose ... ..	G	+	++
Dextrin ... ..	G	+	+

Substance	Growth	Acid production	
		<i>Bacillus lactis-acidi</i> Leichmanni	Bac. No. II
Starch ... ..		—	(+)
Mannit ... ..	G	(+)	+
$\alpha$ -methol-glucoside ... ..	G	—	—
Inulin ... ..	G	(+)	+

Thus this bacillus produces acid from many kinds of carbohydrates but the quantity produced in each case is not much, as shown by titration.

From the above table, it will be seen that this bacillus nearly agrees with *Bac. lactis-acidi* in forming acid from the above named carbohydrates, starch being an exception. But their action upon gelatine are different.

IV. Fermentation products: Acetic and lactic acids, ammonia, and methyl-alcohol were found in the distillate and in the residue of glucose-yeast-water culture, but formic, butyric and succinic acids, fusel oil, ethyl-propyl-isopropyl-and butyl-alcohol, aldehyde, acetone, furfural and indol were not found.

The ratio of the total acid to the non-volatile acid produced in glucose-yeast-water culture, after 10 days at 30° C., was 11'5: 10'5.

V. Conditions of temperature: (a) Optimum temperature for growth lies near 30° C., and no growth is observable below 17° C. and above 52° C. This test was done with yeast-water containing 3% of glucose. (b) Optimum temperature for acid production lies also near 30° C. in the same medium as above. (c) Thermal death-point: Heating to 60° C. for 10 minutes in "Koji"-extract retards the growth of this bacterium very much, an exposure to 70° for 10 minutes kills the cells.

VI. Behavior towards alcohol and lactic acid: 3% of alcohol nearly inhibited the growth, with 7% there was no growth. No development with 0.47% of lactic acid.

VII. Influence of absence of air. This bacillus is aerobic. No growth in anaerobic culture with Buchner's or Botokin's methods.

## PORT II. COCCUS.

### Coccus No. I. (*Pseudosarcina*).

I. Form and Size: The diameter is about  $1.5\ \mu$ . Two, three or cal sarcina-forms are not found. No involution form on "koji"-extractal sarcina-forms are not found. No involution form on "koji"-extract-gelatine after 20 days. Stains with Gram's method. Nonmotile and sporeless.

II. Growth: 1. Solid culture: (a) *Plate cultures*: Bouillon-agar, saccharose-bouillon-agar: Round somewhat elevated, milky white colonies with fatty lustre appear on the surface of medium. The periphery is smooth and the internal part looks homogeneous with a magnification of 170 (one day at  $33^{\circ}\text{C}$ ). The color of the colonies changed into a light yellow tint after 2 days more. Bouillon-gelatine: Forms round, small, yellow colonies. No liquefaction of gelatine (15 days at the room temperature). (b) *Stab-cultures*: "Saké"-agar: A needle like growth on the surface of the medium (24 hours at  $35^{\circ}$  and 6 days more at the room temperature. "Koji"-extract-agar: A faint growth is formed along the needle track and creamy yellowish white colonies appear about the mouth of the stab canal (one day at  $35^{\circ}$ ). Bouillon-gelatine: Similar appearance as in the case of "koji"-extract-agar. No liquefaction after 10 days at the room temperature. (c) *Surface cultures*: Bouillon-agar: Forms a flat creamy, light-yellow growth along the streak. Turbid condensed water has a yellowish white deposit (3 days,  $35^{\circ}\text{C}$ ). Clear condensed water was observed after 20 days. Saccharose-bouillon-agar: A similar growth as in the preceding medium, but the color of the colonies is milky white (3 days at  $35^{\circ}$ ). "Sake"-agar: A creamy white growth along the needle track (24 hours at  $35^{\circ}\text{C}$  and then 2 days

at the room temperature). Bouillon-gelatine: A faint, grayish white growth along the streak after 2 days at the room temperature. After 20 days no liquefaction of gelatine, the color of the colonies being yellow.

2. Fluid culture: "Koji"-extract: After 6 days at 30°C, the liquid is turbid and sediment formed along the wall of the glass tube. Bouillon: Fluid turbid. On shaking, the sediment rises at first as filaments and then scatters like cloud (3 days at 35°). More or less turbid even after 11 days. Wort: Fluid turbid, and some deposits (3 days at 35°). Yeast water: Slightly after turbid 3 days at 30° C. It becomes clear after 10 days. "Saké", diluted "saké" and beer: No growth in any of these media, even after 32 days at 28-33° C. Milk: No coagulation after 25 days at 28-33°C. Artificial solution A: very slight growth, after 10 days at 25-27°M.

IV. Behavior towards carbohydrates, and comparison with the already described microbes.

Substance	Growth	Acid production
Rhamnose ... ..	K	—
Glucose ... ..	G	+
Saccharose... ..	G	+++
Fructose ... ..	G	+
Galactose ... ..	G	+
Maltose ... ..	G	++
Lactose ... ..	G	+
Raffinose ... ..	G	+
Dextrin ... ..	G	+
Inulin... ..	G	+
Starch ... ..		—
Mannit ... ..	G	—
$\alpha$ -methyl-glucoside ... ..	G	—

Thus this coccus forms acid from many kind of carbohydrates, but the quantity produced, in each case is not so great as in Bac. No. I.

According to Lindner<sup>23</sup> almost every sarcina and pediococcus produces more or less lactic acid, but the descriptions for them, as regards the production of acid from carbohydrates generally, are not sufficient. The well known coccus, *Pediococcus acidilacticus* Lindner differs from our coccus No. I, by its inability to produce acid from saccharose, raffinose, dextrin, and inulin. *Sarcina meliflava* Gruber does not cause any turbidity in bouillon-culture, a property which distinguishes it from our Coccus No. I.

IV. Fermentation products: Lactic, succinic-(trace) and butyric acids, fusel oil, ethyl alcohol (trace), ammonia (trace) were found in the distillate and residue of glucose-yeast-water culture, but formic acid, acetic acid, aldehyde, acetone, furfural and indol were not found.

The ratio of total acid to non-volatile acid, in the culture of glucose yeast-water, after 10 days at 30°C, was 13.0: 11.0

V. Conditions of temperature: (a) Optimum temperature for growth lies between 26°C. and 30°C (3% glucose-yeast-water was applied as culture medium). No visible growth below 17°C and above 52°C. (b) Optimum temperature for acid production lies near 25°C (The same medium as above). (c) Heating to 60°C for 10 minutes does not kill all the cells, which die only at 70°C (in bouillon).

VI. Behavior towards alcohol and lactic acid: It grows very well in bouillon containing 7% of ethyl alcohol, but there was no growth with 13%. This coccus grows energetically in saccharose-yeast-water containing 0.17% of lactic acid, but 0.47% of lactic acid inhibits its development.

VII. Relations to the quantity of sugar.

23. Lindner: *Microscopische Betriebskontrolle in den Gärungsgewerben*, 1905, Auf. 4, S. 482.

Percentage of glucose in bouillon ...	2	5	10	15	20	25
Growth after 15 days at 26-29°C ...	+	+	+	+	—	—
	+	+	+	+		
	+	+	+			

VIII. Necessity of air: This coccus grows only in the presence of air.

#### Coccus No. II. (*Pseudosarcina*).

I. Form and Size: The diameter of the cell is about 1  $\mu$ , similar in appearance to Coccus No. I under the microscope. No involution forms are found in bouillon-agar-culture after 2 months. Non-motile and sporless.

II. Growth: 2. Solid culture: (a) *Plate cultures*: Bouillon-agar: Forms small, round, somewhat elevated, creamy, grayish white colonies, with smooth peripheries and homogeneous center under a magnification of 170 (24 hours at 33°C). After two days more at the same temperature the color of the colonies changed into light orange-yellow. Bouillon-gelatine: Forms fine, round, light orange-yellow colonies after 5 days at the room temperature. (b) *Stab-cultures*: "Saké"-agar: A filamentous growth appears along the stab canal, and the surface growth has fatty looking, light orange colonies after 24 hours at 35°C. "Koji"-extract-agar: Good growth with an orange yellow surface (3 days at 35°C). Bouillon-gelatine: A beaded growth appears along the stab-canal, and a round, light orange-yellow colony about the mouth of canal. No liquefaction after 12 days at the room temperature. (c) *Surface cultures*: Bouillon-agar: A milky white colony grows on the streak. Condensed water is clear and some white deposits are formed at the bottom after 20 hours at 33°C. After 20 days, both the growths on the solid medium and at the bottom of the condensed water changed into a light orange-yellow tint. Saccharose-bouillon-agar: Similar growth as above, but the



color of the colony remains milky white even after 20 days. "Saké"-agar: Forms milky white ceveing after 24 hours at 35°C. "Koji"-extract-gelatine: Orange-yellow colonies united together in a chain-form along the streak. No liquefaction of gelatine after 20 days at the room temperature.

2. Fluid culture: "Koji"-extract: Islands appear on the surface of medium, and the liquid is turbid after 5 days at 35°C. Bouillon: Turbid and some islands after 3 days at 35°C. The liquid was turbid even after 16 days and a viscous, flocculent deposit was present. Wort: A faint growth after 3 days at 35°C. Artificial solution A: A very slight growth after 10 days at 30-35°C. Yeast water: Slightly turbid, and a little sediment, which rises as filaments and scatters like cloud, when the tube is shaken. Some islands are on the surface of the liquid. Clear, after 10 days. "Saké", diluted "saké", beer: No growth after 32 days at 28-33°C. Milk: No coagulation after 25 days at 28-33° C.

III. Behavior towards carbohydrates, and comparison with the already described cocci.

Substance	Growth	Acid production
Rhamnose ... ..	G	—
Glucose ... ..	G	+
Saccharose... ..	G	+
Fructose ... ..	G	+
Galactoss ... ..	G	+
Maltose ... ..	G	+
Lactose ... ..	G	—
Raffinose ... ..	G	—
Dextrin ... ..	G	(+)
Inulin ... ..	G	—
Starch ... ..		—
Mannit ... ..	G	—
α-methylg.-lucoside ... ..	G	—



Thus this microbe produces acid from comparatively few carbohydrates, and the quantity produced is also small. This coccus agrees with *sarcina aurantiaca* in the formation of orange-yellow color but the coagulation of milk and the liquefaction of gelatine by the latter distinguishes from *Coccus* No. II.

IV.—Fermentation products: Butyric-(trace) and lactic acids, methyl-lactate, fusel oil, methyl-alcohol and ammonia (trace) were found in a glucose-yeast-water culture, but formic acid, acetic and succinic acid, aldehyde, acetone, furfural and indol were not found. Moreover the distillate had an odor like that of amyl-valerate, although it was not tested. The ratio of total acid to non-volatile acid produced in the culture of yeast-water-glucose, after 10 days at 30°C, was 9.5:8.0.

V. Conditions of temperature: a. Optimum temperature for growth lies between 26° and 30° C., and growth is not apparent below 17° or above 52° in 3% glucose-yeast-water. b. Optimum temperature for acid production is below 30°C in the same medium as above. c. Thermal death point lies between 60° and 70°C, heating for 10 minutes in bouillon.

VI.—Behavior towards alcohol and lactic acid: It grows in bouillon containing 7% of ethyl-alcohol, but there is no growth with 13 per cent. of alcohol in the same medium. No growth with 0.47% of lactic acid.

VII. Relation to the quantity of sugar:

Percentage of glucose in bouillon ...	2	5	10	15	20	25
Growth after 15 days at 26-29°C. ...	+	+	+	+	—	—
	+	+	+			

VIII. Necessity of air: Both with Buchner's and Botokin's methods this coccus makes a slight growth in bouillon.

### COCCUS NO. III. (SARCINA).

I. Form and size: The cell is round and its diameter is about

1.2  $\mu$ . Usually two or four cells are united together in a chain or a cross form but rarely in a sarcina-form in bouillon. Non-motile and sporless.

II. Growth: 1. Solid culture: a. *Plate cultures*: Saccharose-bouillon-agar: Forms a round, milky white colony on the surface of the medium. Its periphery is smooth, and the internal parts look homogeneous under the microscope after 2 days at 35°C. b. *Stab-cultures*: "Saké"-agar or "koji"-extract-agar: A spare growth along the stab canal, with a flat surface growth of a creamy nature and white color (24 hours at 35°C and then 6 days at the room temperature). Bouillon-gelatine: Both the growths in the stab canal and on the surface of the medium are feeble. No liquefaction of gelatine after 18 days at the room temperature. c. *Surface cultures*: Bouillon-agar: Round milky white colonies form a chain along the streak, and the condensed water is turbid, with a white deposit after 2 days at 35°C. The liquid becomes perfectly clear after 17 days. Saccharose-bouillon-agar or "saké"-agar: Similar appearance is in the foregoing medium the temperature and time being the same.

2. Fluid cultures: "Koji"-extract: Fluid is turbid, and a white sediment appears after 4 days at 35°. On shaking the deposit rises as filaments and diffuses immediately like cloud. Bouillon: Fluid is turbid after 3 days at 35°C. Yeast water: Slightly turbid after 10 days at 28-33°C. "Saké," diluted "saké," beer: No growth after 32 days at 28-33°C. Milk: No coagulation after 25 days at 33°C.

III. Behavior towards carbohydrates, and comparison with the already described cocci.

Substance	Growth	Acid production
Rhamnose ... ..	K	—
Glucose ... ..	G	++
Saccharose ... ..	G	+++
Fructose ... ..	G	++
Galactose ... ..	G	+
Maltose ... ..	K	—
Lactose ... ..	G	—
Raffinose ... ..	G	(+)
Dextrin ... ..	G	+
Inulin .. ...	G	+
Starch ... ..		—
Mannit ... ..	G	—
$\alpha$ -methyl-glucoside ... ..	G	—

Comparison: *Sarcina pulchra* Henrici, and *S. nivea* Henrici grow only in the presence of air, thus differing from our Coccus No. III. This coccus agrees in many of its properties with Saito's<sup>9</sup> *Sarcina Hamaguchiae*, but the differential character is that my coccus develops in bouillon while *S. Hamaguchiae* does not.

IV. Fermentation products: Butyric acid (trace), lactic acid, ethyl alcohol, ammonia, and methyl alcohol (trace) were found from the distillate and residue of glucose-yeast-water culture, but formic acid, acetic acid, fusel oil, aldehyde, acetone, furfural, succinic acid, and indol were not found.

V. Conditions of temperature: a. Optimum temperature for growth lies between 26° and 30°C, and growth is not apparent below 17° and above 52°C in glucose-yeast-water. b. Optimum temperature for acid production is nearly the same as that for growth. c. Thermal death

point lies between 60° and 70°C, heating being done for 10 minutes in bouillon.

VI. Resistance against alcohol, and lactic acid: An energetic growth takes place in bouillon containing 7% of alcohol, but with 13% it ceases. It grows well in neutral-yeast-water containing 10% of saccharose and 0.17% of lactic acid, but 0.47% of lactic acid inhibits the development of this microbe.

VII. Necessity of air: Both with Buchner's and Botokin's method *it grows as well as in open air.*

### Summary.

1. Two of the isolated microbes are bacilli; *B. Aderholdii* var. *moto*, *B. lactis acidii* Leich. var. *moto*, and easily distinguishable from each other by stab-cultures. The others are cocci, and distinguishable from each other by surface growths on bouillon-agar. *Bac. lactis acidii* Leichm. var. *moto* is *motile*, but the others are not.
2. None of the four species grows in "saké" or beer, and is therefore not injurious to them. They do not produce any change in milk and do not liquefy gelatine, except *Bac. No. II.* (*Bac. lact. acidii*: Leichm. var. *moto*). They grow well on solid media with the exception of *Bac. Aderholdii* var. *moto*, which does not. The growth of all four is almost entirely inhibited by 0.47% of lactic acid in saccharose-yeast-water.
3. The fermentation products in yeast-water-glucose, especially the production of acid from carbohydrates differs according to the species. In all cases, lactic acid, small quantity of volatile acid, and ammonia were found, but no aldehyde, furfural, indol or gases. Some slimy substances were produced by *Bac. No. II.* *Bac. lactis acidii* Leichmanni var. *moto*, and *Coccus No. II.* All

four produce a relatively small quantity of acid, Bac. No. I. (Bac. Aderholdi var. moto) producing 0.9% (as lactic acid) in "koji"-extract.

4. Bacillus No. II. and Coccus No. I. grow only in aerobic cultures, but Bac. No. I. (Bac. Aderholdi var. moto) and Coccus No. III. develop well even in anaerotic culture, while Coccus No. II. Bac. lactis-acidi-Leichmanni-var. moto. grows slightly in anaerobic cultures.

In conclusion my thanks are due to Prof. T. Takahashi for his kind advices given during the progress of the work.

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## Note on Yeasts from Quince Liquor.

BY

H. Itō.

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With Plate XV.

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Quince liquor<sup>1</sup> was prepared from 45 litres of juice obtained by pressing 47 k.g. of quince. Before and during the fermentation microscopic examination for yeast was made on the mash. The yeasts isolated are briefly described below:—

*Sacch. apiculatus*. (From both fruits and mash)

I. Form and size:—Citron shaped; commonly  $5 \times 2.5 \mu$ , larger ones

1. This liquor has the following composition:—

Sp. gr. (at 18°C) ... ..	1.021
In 100 c.c.	
Alcohol (vol. percent) ... ..	6.4
Extract ... ..	8.112 grams.
Sugar (as glucose) ... ..	3.888 "
Glycerin ... ..	0.340 "
Total acid (as tartaric acid) ... ..	0.886 "
Volatile acid (as acetic acid) ... ..	0.617 "
Non-volatile acid (as tartaric acid ester) ... ..	2.698 "
Volatile ester (as acetic ester) ... ..	0.084 "
Non-volatile ester (as neutral tartaric diethyl ester) ... ..	2.600 "
Ash ... ..	0.286 "

8.7×5  $\mu$  smaller ones 3.7×1.2  $\mu$ . The daughter cell separates easily from the mother cell.

II. Contents of cells:—They are granular, clear and rich in vacuoles which generally contain refractive and revolving bodies or granules. Spore was not formed.

III. Fluid culture:—Culture in “kōji”-extract kept at 30° C for 24 hours: Fluid was turbid with sediments. When it was shaken, the sediments assume the form of threads and diffuse in fluid. Films were not formed on “kōji”-extract, yeast-water and Hayduck’s solution kept at 30° C for two months.

IV. Solid culture:—

1. Plate culture on “kōji”-extract-agar kept at 25-30°C for a day. Colony was round, slightly elevated, lustrous, waxy and gradually turned somewhat greyish white. The margin looked smooth under the microscope.
2. Stab culture:—(a) On “kōji”-extract-gelatine kept at 10-15° C for 5 days. Formed feeble growth along the stab canal and feeble, round, white, waxy, growth around the mouth of the canal. (b) On “kōji”-extract-agar kept at 14-16°C for 5 days: Formed feeble growth along the stab canal and round, waxy, dirty greyish growth around the mouth of the canal.
3. Streak culture: (a) On “kōji”-extract-gelatine kept at 10-15° C for 5 days: Feeble growth was flat, waxy, along the track and the margin showed fine radiating lines. (b) On “kōji”-extract-agar kept at 14-16°C for 5 days: Growth along the track was feeble, flat, moist, somewhat yellowish grey and condensed water was clear, with sediments.
4. Gigantic colony: On “kōji”-extract-gelatine kept at the room temperature (at 10°-14° C) for one month. It was dish like, moist, lustrous, greyish, waxy, with wavy and radiating margin.



V. Behavior toward different sugars: With Lindner's method and Einhorn's fermenting tube.

Sugars	Fermentation	
	Investigated apiculatus	Sacch. apiculatus Rees.
<i>d</i> -Fructose .. .. .	+++	+++
Glucose ... .. .	++	++
<i>d</i> -Mannose ... .. .	+	+++
Saccharose .. .. .	—	—
Maltose ... .. .	—	
Galactose ... .. .	—	+
Rhamnose . . . . .	—	—
Dextrin ... .. .	—	—

Thus this apiculatus differs from Sacch. apiculatus, Rees. by fermenting d-galactose.

VI. Experiment on assimilation; according to Beijerinck's method:

2. +++ denotes comparative strong fermentation.
- ++ denotes tolerable strong fermentation.
- + denotes feeble fermentation.
- denotes no fermentation.

Substance as source of nitrogen.	Assimilation
Peptone ... ..	+
Asparagine... ..	+
Ammonium ... ..	—
Sodium nitrate... ..	—
Potassium nitrite ... ..	—
Substance as source of carbon.	Assimilation.
Saccharose... ..	—
Maltose ... ..	+
Glucose ... ..	+
Dextrin ... ..	+
Galactose ... ..	+
Lactose ... ..	—

VII. Degree of fermentation: In "kōji"-extract.

Apparent fermentation index ..... 5.8

Actual fermentation index ..... 5.2

VIII. Assimilation of amino-acid: Culture in "kōji"-extract<sup>3</sup> kept at 30°C for 25 days. It consumed 0.0767 g. in 100 c.c. of "kōji"-extract. -

IX. Conditions of temperature: Optimum temperature was 30° C. 55° C for 30 min. was not sufficient to destroy its vitality, but it was killed at 60° C for 30 min.

X. Fermentation products: Ethyl-, methyl-, amylalcohol and aldehyde were found in the distillate of "kōji"-extract culture after 7 days at 30° C and lactic acid in the residue, but acetic-, butyric-, propionic acid and acetone were not found. From the above description we see that this yeast belongs to *Sacch. apiculatus* Rees.

3. 100 c.c. of the "kōji"-extract contained 0.4631 g. amino acid calculated as glycocoll.

*Torula*. (From both fruits and mash.).

I. Form and size: Round and somewhat elliptical; generally 6  $\mu$  in diameter, very large ones 7.5  $\mu$  small ones 1  $\mu$ . Generally one or two cells combined but three cells combined were rare.

II. Contents of cells. They were homogeneous, clear and rich in vacuoles which sometimes contained refractive and revolving granules. Glycogen reaction was positive.

Spore was not formed, but it formed large fatty bodies, which sometimes entirely replaced the contents.

III. Mode of growth: Generally budding, but it was noted in droplet cultures of "kōji"-extract that a daughter cell sometimes hung on to a large burst mother cell (with diameter of 7.5  $\mu$  and with a cell-membrance with 1.5  $\mu$  thick.

IV. Fluid culture:

1. Culture in "kōji"-extract kept at 25° C for 4 days: Grew well in the surface part of fluid with heavy sediment. When it was shaken, the sediment assumes the form of threads which easily broke into pieces, but diffused in the fluid with difficulty. Formed ring after 3 days at 30° C, but no film was formed in "kōji"-extract culture kept at 30° C for one month more.
2. Culture in milk kept at 30° C for one day: Formed coagulum and gradually dissolved it.

V. Solid culture:

1. Plate culture on "kōji"-extract-agar at 25-30° C for one day: Colony was round, elevated, waxy, slightly rose coloured, its periphery was smooth under the microscope.
2. Stab culture: (a) In "kōji"-extract-gelatine kept at 10-15° C for 4 days: Grew faintly along the stab canal and formed dry, lightly rose coloured, gloomy, waxy, elevated growth and the

margin was waxy. (b), In "kōji"-extract-agar kept at 14-16° C for 3 days: It grew faintly along the stab canal and formed moist, elevated, somewhat reddish brown growth along the mouth of the stab canal and the margin was waxy.

3. Streak culture: (a) In "kōji"-extract-gelatine kept at 10-15° C for 3 days. Growth along the track was dry, gloomy, yellowish brown, elevated, afterward it gradually changed to brown and the margin was waxy. (b) In "kōji"-extract-agar at 14-16° C for 4 days: Along the track it formed elevated, moist, lustrous yellowish brown growth and condensed water was clear but contained sediments.
4. Gigantic colony: In "kōji"-extract-gelatine kept at 10-14° C for 25 days. It was elevated, but the centre was concave and the margin showed concentric rings and faint streams. Its periphery was waxy. Root like growth<sup>4</sup> was formed under the concave part of the colony. Gelatine was liquefied after 30 days and the part was acid.

VI. Behavior toward different sugars: With Lindner's method and Einhorn's fermenting tube.

Sugar	Fermentation	
	Investigated <i>Torula</i>	<i>Torula pulcherrima</i> Lindner
d-Fructose... ..	+++	+++
Glucose ... ..	+++	+++
d-Galactose ... ..	++	+
d-Mannose... ..	+	++
Saccharose ... ..	?	—
Maltose ... ..	—	—
Rhamnose... ..	—	—
Dextrin ... ..	—	—

4. Will, Central Blatt für Bakt. XXVIII S. 401.

Thus this torula behaves almost same as torula pulcherrima.

VII. Degree of fermentation: In "kōji"-extract.

Apparent fermentation index ..... 6.0

Actual fermentation index ..... 5.3

VIII. Consumption of amino-acid: Culture in "kōji"-extract kept at 30°C 25 days. It consumed 0.0767 g. amino-acid as glycocoll in 100 c.c. of "kōji"-extract.

IX. Experiment on assimilation: According to Beijerinck's method.

Substances as source of nitrogen	Assimilation
Peptone ... ..	+
Asparagine .. ...	+
Ammonium ... ..	+
Sodium nitrate ... ..	+
Potassium nitrite ... ..	—
Substances as source of carbon	Assimilation.
Saccharose ... ..	+
Maltose ... ..	+
Glucose ... ..	+
Dextrin ... ..	+
d-Galactose ... ..	+
Lactose ... ..	—

X. Conditions of temperature: Optimum temperature lay 30° C. 55° C for 30 min. was not sufficient to destroy its vitality, but it was killed at 60° C for 30 min.

XI. Fermentation products: Ethyl-, methyl-, amyl-alcohol and aldehyde (trace) were formed in the distillate of "kōji"-extract culture after 7 days at 30° C and lactic acid in the residue, but acetic-, butyric-, propionic acid, and acetone were not found.

Thus this yeast belongs to *Torula pulcherrima*.

I express my great thanks to Prof. T. Takahashi for the advices given during this work.

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Explanation of Figures.

Plate XV.

Fig. 1. A<sub>1</sub> The "kōji"-extract-gelatine culture of apiculatus.

A<sub>2</sub> The "kōji"-extract-agar culture of apiculatus.

T<sub>1</sub> The "kōji"-extract-gelatine culture of Torula.

T<sub>2</sub> The "kōji"-extract-agar culture of       ,,

Fig. 2. Apiculatus: 1200/1 "kōji"-extract culture at 30°C for 24 hours.

Fig. 3. Torula; 900/1. "kōji"-extract culture at 30°C for 24 hours.

Fig. 4. Gigantic Colony of Torula on "kōji"-extract-gelatine for 25 days at room temperature.

Fig. 5. Gigantic colony of Torula on "kōji"-extract-gelatine for 50 days at room temperature.

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Fig. 1.



Fig. 2.

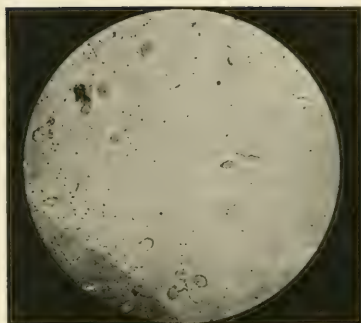


Fig. 3.



Fig. 4.

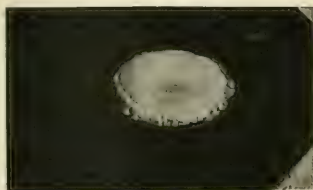


Fig. 5.







## Note on Yeasts of "Sho-yu"-mash.

BY

T. Mitsuda.

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With Plate XVI.

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This short report is based on work carried on parallel with that of the carbohydrates of "sho-yu" mash, the result of which was published a former number of this journal<sup>1</sup>. At that time the only published report on the microorganisms of "sho-yu" mash was that of K. Saito who described one variety of yeast and the name of *Saccharomyces Soja* but recently T. Nishimura published a long paper in the "Nogaku-Kai-Kaiho"<sup>2</sup> and described three varieties of yeast isolated from "sho-yu"-mash.

Three samples were used for the purpose of isolation, one from Noda, one from Tatsuno, and the other from Handa. 8 different varieties of yeast, 3 bacilli and 3 mould fungi were obtained from them. 3 varieties from Noda sample, 1 variety from Tatsuno sample, 1 variety from Handa samples, besides a red yeast and two film forming yeasts. However only 5 varieties of yeast were investigated.

### Method of Isolation.

As culture medium for the isolation of microorganisms by plate culture sterilised kōji-extract-agar was used; culture temperature 28° C.; purification by droplet culture after Lindner.

1. Vol. 1. No. 1. 1903.

2. Journal of the scient, Agricu. Society. (Japanese).

I will describe briefly the characteristics of these five:—

### The First Variety.

- (a) **Form and Size:** Mainly spherical, sometimes more or less elongated, and 6-9  $\mu$  in size in koji-extract. The contents very dull, granular and rich in glycogen.
- (b) **Growth:** Colonies are greyish-white, very waxy and bright, round and elevated on the surface of plate-culture; waxy, bright, round and smooth in the inner part; periphery either smooth or more or less zig-zag shaped (Cf. Fig. I.). *Streak-culture:* a white, bright, waxy and elevated growth along the track, with wavy surface, and streamings on margin. *Stab-culture:* white and folded at the mouth of stab-canal, monili form with gas bubbles along the line. Gigantic-colony: mealy, white, elevated, very folded and dried appearance after 30 days at the room temperature (Cf. Fig. 6). The growth in koji-extract, wort, yeast-water and other nutrient media is very favorable. Yeast ring appears after 3 days at 30 C., developing into a greyish thin film over the surface of the fluid.
- (c) **Spore-formation:** This variety does not form spores on gypsum block at 25-35 C. after 3 days.
- (d) **Assimilation of Carbon and Nitrogen:** This experiment was made with Mayer's method and was repeated three times with the same fluid to exclude the possible influence of former nutrition. This variety assimilates carbon from almost all the carbohydrates and some organic salts, but not from ethyl alcohol (Cf. Table I). Nitrogen is assimilated from peptone.

asparagine, ammonium salt and nitrate and also perhaps from nitrite.

- (e) Behavior towards organic acids: It can grow in koji-extract containing 2.5% succinic or 1.5% acetic, but not in 1.75% acetic acid.
- (f) Conditions of Temperature: Optimum temperature for growth lies at 28-32 C. and growth is disturbed at 25 C. and vegetative cells die at 60 C. in 4-5 minutes.
- (g) Fermentative Faculty: It was determined with Lindner's method. It ferments sucrose, maltose, raffinose, dextrose etc. but not lactose etc. (Cf. Table 2). Koji-extract (12° B) sinks to 3.5° B after fermentation, giving the apparent fermentation coefficient of 70.8%.
- (h) Alcoholic production in koji-extract containing various quantities of NaCl during two weeks at 28-30 C. is greatest in 5% NaCl, i.e. 5.25% of ethyl alcohol., 20% NaCl disturbs its growth to a high degree (Cf. Table 3).

### The Second Variety.

- (a) Cells are spherical or oval, 5-8  $\mu$  in size, contents homogenous and bright, vacuoles large, stains brownish red with I+KI solution.
- (b) On plate culture: White greyish, elevated and very folded colonies on surface; round and smooth in inner part as in the case of the first variety (Cf. Fig. 2). *Streak culture*: greyish white, elevated and dull along the track, flat and smooth in the centre and wavy on both sides. The streak culture on koji-extract-agar differs distinctly from that of the first variety. *Slab culture* is very similar to the first variety. *Gigantic colony* is white elevated and folded, with wet surface (Cf. Fig. 7). In the above mentioned fluid media it grows

very favorably, forms a white greyish film on the surface of media after long time, but very quickly in the media containing some NaCl.

- (c) Spore-formation was not observed on gypsum block at any temperature as in the first variety.
- (d) Faculty of assimilation of carbon and nitrogen from various sources comes very closely to the first; also carbon is assimilated pretty well from ethyl-alcohol (Cf. Table 1).
- (e) This variety can grow in koji-extract containing 2.5% succinic acid or 1.25% acetic, but not in 1.75% acetic acid.
- (f) The optimum temperature for growth lies at 30° C. at 34° C. growth is much retarded.
- (g) Fermentative faculty closely resembles that of the first variety (see Table 2). Apparent fermentation coefficient 70.8%.
- (h) Amount of alcohol produced in koji-extract containing NaCl varies with the amount of NaCl added. 5% NaCl in koji-extract gives the most favorable result i.e. 5.25% ethyl alcohol; 20% NaCl gives 3.78% alcohol (Cf. Table 3).

### The Third Variety.

- (a) Spherical form: 5-8  $\mu$  in diameter, bright and homogeneous.
- (b) On koji-extract-agar plate, it forms bright greyish white, round and elevated colonies, with smooth surface and periphery (Cf. Fig. 3). *Streak culture*: A white, waxy,, bright and elevated growth, the folds on surface finer and less than that of the foregoing two varieties, layers and canals at the center of growth, periphery toothed. *Slab culture*: A white irregular, highly elevated and smooth growth at the mouth of stab canal, but in inner part it is moniliform with gas bubbles.  
*Gigantic colony*: The development of the gigantic colony

is very similar that of the second (Cf. Fig. 8). It grows in many nutrient media forms yeast ring after 5 days at 28° C., but does not form any complete film on the surface of media even after 15 days at 25° C. It makes most favorable growth in koji-extract or wort containing 10% NaCl and forms a grayish film after a long time on the surface. In droplet culture a single cell grows quickly and makes a large band. Spore formation on gypsum block was not observed.

- (c) This variety can assimilate carbon from almost all the carbohydrates and some organic salts, but not from ethyl-alcohol. Also, nitrogen from various forms of nitrogenous compounds except nitrite (Cf. Table I).
- (d) It can grow in 2.5% succinic acid and 1.25% acetic acid in koji-extract, but not in 1.5% acetic. Its development is profoundly disturbed by 10% ethyl-alcohol in koji-extract. Its apparent fermentation coefficient in koji-extract is 73%.
- (e) It can develop favorably at a comparatively high temperature i.e. its optimum temperature for growth lies at 30-32C. About fermentative faculty and ethyl alcohol production in NaCl solution of koji-extract of this variety, see tables 2 and 3.

#### The Fourth Variety.

- (a) Spherical or oval cells, mean size 5-8.5  $\mu$ , sometimes larger cells (i.e. "Dauerzellen" 10  $\mu$ .) are found in yeast ring. Vacuoles large, cells bright, granular, and rich in glycogen.
- (b) On plate culture are found white grayish, elevated, irregular, and dull colonies, their surface is wavy and elevated especially at the center, with round and smooth periphery in inner part of the medium. (Cf. Fig. 4). *Streak culture*: Very similar to that of the second variety but whiter in colour. *Slab*

*culture*: White, dried and elevated growth is found at the mouth of the stab canal. *Gigantic colony*: very similar to that of saké-yeast, white, elevated and foded in the form of a crater. (Cf. Fig. 9). It grows in various nutritive media. No film is found at 25° C. during 15 days, but yeast ring is formed after 3 days 30 C: Most remarkable development was observed 10% NaCl solution in koji-extract. By droplet culture after Lindner a single cell developes very quickly into a large band.

- (c) This variety assimilates carbon from many carbohydrates and some organic salts, nitrogen from various form of its compounds. (Cf. Table 1.)
- (d) It grows in 2.5% succinic or 1.5% acetic acid, but not in 2% acetic acid, in koji-extract. Its growth is retarded by 10% alcohol and no further production of alcohol takes place. Its optimum temperature for growth lies at 30-32° C.
- (e) The fermentation faculty of sugars resembles that of the former variety, and the apparent fermentation coefficient is 66.9% Production of ethyl-alcohol in koji-extract was more or less disturbed when NaCl. was increased (Cf. Tables 2 and 3).

### The Fifth Variety.

- (a) Cells spherical or oval, mean size 4-8  $\mu$ , not bright. One or more moving granules in the vacuoles (or "each vacuole").
- (b) On plate culture of koji-extract-agar, round, yellowish-white, elevated, smooth, waxy, bright colonies appear on the surface, with smooth periphery. (Cf. Fig. 5). *Streak culture* is yellowish-white, waxy, elevated and bright. Surface and periphery smooth. *Slab culture*: A yellowish-white round, elevated growth, smooth and bright at the mouth of stab canal and many small colonies with gas bubbles along the canal.

*Gigantic colony.* Yellowish white, elevated, smooth, creamy bright colony, with smooth periphery. Growth in the fluid media mentioned above, is favorable. The film formation was not observed at any temperature, even in NaCl containing koji-extract. In droplet culture a single cell develops very quickly into a large band.

- (c) On gypsum block spore formation was not observed at any temperature.
- (d) Assimilation of carbon and nitrogen from various sources and resistance against organic acids similar to those of the foregoing varieties. The optimum temperature of growth lies at 28-30 C, but it is comparatively resistant against high temperature. It ferments dextrose, saccharose, maltose and others, but not lactose etc. *It is curious that this yeast grows well in wort, but not in koji-extract.* The apparent fermentation coefficient is 37.14%. NaCl has a deterrent action on the production of ethylalcohol i.e. 2% NaCl in wort gives only 1.60 ethyl alcohol, while the control gives 3.57% (Cf. Table 3).

### Summary.

It is highly probable that the 5 varieties described, differs from *Saccharomyces Soja*, Saito, by fermenting Sucrose and raffinose, while his yeast does not ferment either. But the third variety somewhat resembles to Saito's, fermenting these two sugars very feebly. While, *Sacch. Soja* forms spores only in the cells of the yeast ring, it does not form on gypsum block, but in our yeast varieties they always absent.

TABLE I. SOURCE OF CARBONE AND NITROGENE

	Variety I.	II	III	VI	V
Cane sugar ... ..	+++	+++	+++	+++	+++
Dextrose ... ..	+++	+++	+++	+++	+++
Galactose .. ...	+	+ trace	++	+	++
Lactose ... ..	+	++	trace	+	+
Fructose ... ..	+++	+++	+++	+++	+++
Mannit ... ..	+++	+++	+	+	+++
Dextrin ... ..	++	+++	+	++	+++
Arabinose ... ..	+	trace	—	+	+
Glycerine ... ..	++	++	+	+	+
Inulin ... ..	++	++		+	+
Na-acetate ... ..	++	+	trace	+	++
Na-lactate ... ..				+	+
Am-tartrate ... ..	+++	+++	+++	+++	+++
Alcohol ... ..	—	+			+
Am-carbonate ... ..	++	+	+++	+	+++
Am-tartrate ... ..	+++	+++	+++	+++	+++
Na-nitrate ... ..	++	++	++	+++	+++
Nitrite ... ..	+?	—	—	trace	—
Am-sulphate ... ..	+++	+++	+++	+++	+++
Asparagine ... ..	+++	+++	+++	+++	+++
Peptone ... ..	+++	+++	+++	+++	+++



TABLE II. FERMENTATION FACULTY.

	Variety I	II	III	VI	V
Sucrose ... ..	+++	+++	+	+++	
Dextrose ... ..	+++	+++	+++	+++	
Fructose ... ..	+++	+++	+++	+++	
Maltose ... ..	+++	+++	+++	+++	
Galactose... ..	—	—	—	—	
Mannose ... ..	++	+++	+++	++	
Lactose ... ..	—	—	—	—	
Raffinose ... ..	+	+	+?	++	
Inulin ... ..	—	—	—	—	
Dextrin ... ..	++	+	+	+	
Glycerine ... ..	—	—	—	—	
Arabinose ... ..	—	—	—	—	

TABLE III. AMOUNT OF ALCOHOL PRODUCED IN NaCl SOLUTION OF KOJI-EXTRACT OR WORT.

Variety I.

% or NaCl	Sp. Gr. of Distillate	Alcohol in Vol. %
0.0	0.9930	4.88
5.0	0.9925	5.25
10.0	0.9930	4.48
15.0	0.9540	4.18
20.0	0.9955	3.07

## Variety II.

% of NaCl	Sp. Gr. of Distillate	Alcohol in Vol. %
0.0	0.9925	5.25
5.0	0.9925	5.25
10.0	0.9930	4.88
15.0	0.9935	4.51
20.0	0.9945	3.78

## Variety III.

% or NaCl	Sp. Gr. of Distillate	Alcohol in Vol. %
0.0	0.9920	5.58
5.0	0.9920	5.58
10.0	0.9920	5.58
15.0	0.9930	4.88
20.0	0.9950	3.42

## Variety IV.

% of NaCl	Sp. Gr. of Distillate	Alcohol in Vol. %
0.0	0.9930	4.89
5.0	0.9935	4.58
10.0	0.9935	4.51
15.0	0.9940	4.18
20.0	0.9955	5.07

Variety V.

% of NaCl	Sp. Gr. of Distillate	Alcohol in Vol. %
0.0	0.9955	3.57
2.0	0.9965	1.160
5.0	0.9980	1.34
10.0	0.9985	1.00
15.0	—	—
20.0	—	—

## Explanation of Figures.

## Plate XVI.

Figs. 1-5, The growth of colonies of yeast on koji-extract-gelatine plate at 28C.

Figs. 6-9, The growth of gigantic colonies of yeast on koji-extract-gelatine 30 days at room temperature.

Fig. 1, Variety 1.

Fig. 2, " 2

Fig. 3, " 3.

Fig. 4, " 4.

Fig. 5, " 5.

Fig. 6, " 1

Fig. 7, " 2

Fig. 8, " 3.

Fig. 9, " 4.



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Fig. 1.

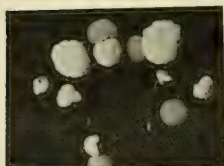


Fig. 2.

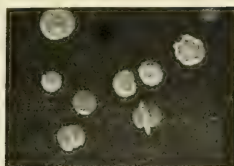


Fig. 3.

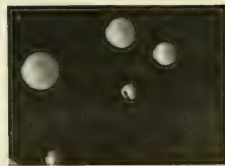


Fig. 4.



Fig. 5.

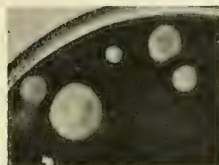


Fig. 6.

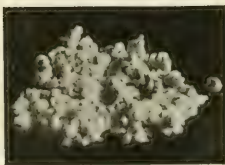


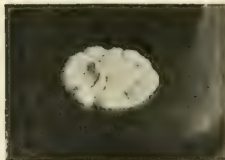
Fig. 7.

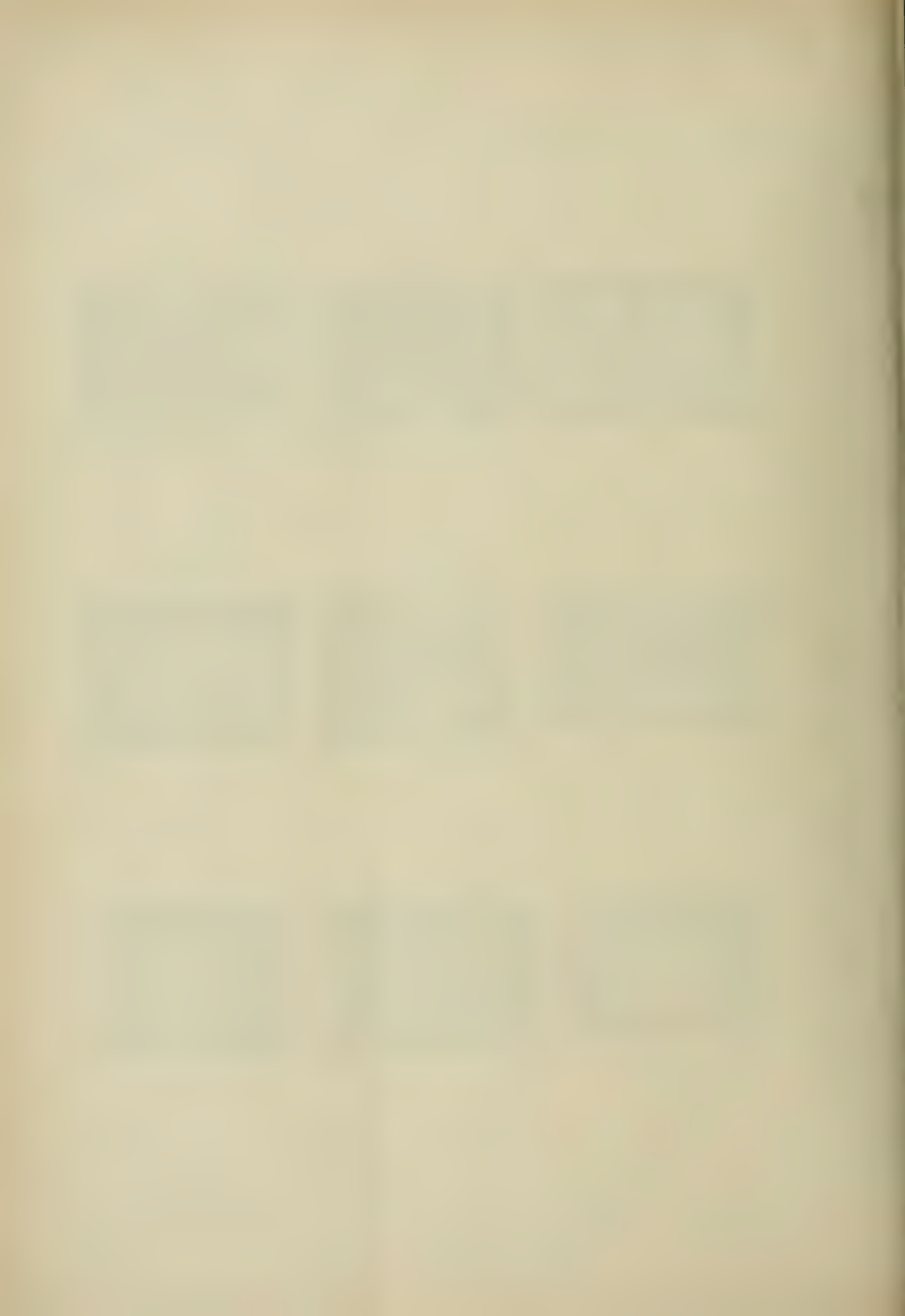


Fig. 8.



Fig. 9.





## Zwei neue *Aspergillus* Arten aus „Katsuobushi.“

VON

M. Yukawa.

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Mit Tafeln XVII und XVIII.

In Japan ist die Verwendung des getrockneten Fisches als Volksernährung schon von alters her bekannt, und vor allem wird getrockneter Bonit (*Gymnosarda pelamis*) im Haushalt stark konsumiert. Zwecks Herstellung des getrockneten Tunfisches, „Katsuobushi“ genannt, wird zunächst das in längliche Stücke geschnittene Fleisch des Tunfisches in einem Kessel gekocht und, wenn es abgekühlt ist, in ein Fass eingepackt, worauf allmählich Schimmelpilze auf dem Fleische wachsen. Das mit Schimmel bedeckte Fleisch wird in der Sonne getrocknet, und nachher die Schimmelpilzdecke abgeputzt. Diese Behandlung, d.h. die wechselseitige Verpackung und Trocknung des Fleisches, wird so lange wiederholt, bis die Haltbarkeit desselben vollständig sicher ist. Im Handel ist das getrocknete Fleisch, das mit grünlichen Schimmelpilzen bedeckt ist, teurer als das mit gelben Pilzen.

Trotz vieler Untersuchungen zur Verbesserung des Trocknungsverfahrens des Tunfisches existiert bislang keine Angabe über die mikroskopische Erforschung, und daher halte ich für gut, diese Studie zu veröffentlichen. Aus verschiedenen von mir untersuchten „Katsuobushi“ Proben habe ich stets zwei Arten des *Aspergillus*, zuweilen ausserdem *Aspergillus albus*, *Verticillium glaucum*, *Penicillium glaucum*, *Mucor racemosus* und andere Pilze isoliert. Von diesen beiden *Aspergillus* Pilzen wird die grüne Art vorzüglich aus der teureren Ware isoliert,

während die mit bernsteinartiger Farbe aus den geringeren Sorten der Proben erhalten wird.

Es ist hoch interessant zu finden, dass diese beiden Species eine stark peptonisierende Kraft besitzen, sie sind also unbekannte neue Arten; für die bernsteinartige Sorte schlage ich den Namen *Aspergillus melleus* vor, für die grüne Art den Namen *Aspergillus gymnosardae*.

### I. *Aspergillus melleus* nov. spec. (Hierzu Taf. XVII, Fig. 1-7).

Es ist bemerkenswert, dass diese Art nicht nur auf verschiedenen Substraten reichlich Sklerotien bildet, sondern auch die sekundären Sterigmen oft zu langgestreckten Schläuchen auswachsen lässt, und ferner die Eiweisstoffe sehr kräftig verflüssigt.

#### A. Morphologisches.

Diese Art bildet sowohl auf festen wie auf flüssigen Substraten anfangs weisse, bald bernsteinartige Deckne, bei älteren Kulturen geht die Färbung in weisslich-braungelb über. Die vegetativen Hyphen sind farblos, hell, zart und mit Querwänden versehen; ihr Durchmesser ist 2-5  $\mu$ . Der Konidienträger entwickelt sich von den Mycelien aus zu einem Seitenzweig und erweitert sich zu einem Köpfchen. Der Stiel nimmt in der Decke von der Blase nach dem Köpfchen allmählich zu, um schliesslich in eine Blase überzugehen. Die Blase steht aufrecht oder nickend auf dem Stiel und ist in der Regel kugelförmig (selten keulig), aber nicht scharf gegen den Stiel abgesetzt. Die ausgewachsenen Konidienträger sind ziemlich stattlich (0,7 - 1 mm.), und ihre Breite 7-25  $\mu$ ; die älteren Träger sind mit ein oder mehreren Septa versehen und zeigen gelb-braune Färbung. Die sehr dünne Wand (0,5-0,7  $\mu$  dick) des Stieles ist glatt und farblos, beim Alterwerden aber oft rauh warzig. Die Blase, 20-50  $\mu$  im Durchmesser haltend, ist allseitig mit dicht gedrängten, radiär ausstrahlenden, stets verzweigten Sterigmen besetzt. Die primären



Sterigmen sind keulig und haben wieder je 3-4 längliche sekundäre Sterigmen; ihre Länge ist  $10-22\ \mu$  und ihre Breite  $2,5-4\ \mu$ . Die sekundären Sterigmen sind kegelförmig,  $10-16\ \mu$  lang (zuweilen bis zu  $30\ \mu$ ) und  $1-2\ \mu$  breit. Missbildungen, z. B. abnormes Auswachsen der Sterigmen, Gabelung des Stieles, unregelmässige Verzweigung der Trägerspitze unter Fortfall der Blasenentwicklung u.a., sind nicht selten bei dieser Art.

Die nicht kurzen Konidienketten sitzen an der Spitze der sekundären Sterigmen. Die Konidien sind klein, glatt, kugelförmig (oder selten ellipsoidisch) und messen  $2,5-4$  im Durchmesser.

Diese Art bildet reichlich sowohl auf festen wie auf flüssigen Substanzen kugelige, harte Sklerotien (Grösse  $0,4-0,7$  mm. Dm.)

Die Sklerotien entstehen durch Verflechten und Verwachsen gleichwertiger Hyphen und die Rinde derselben ist gelbbraun, aber das Mark farblos. Askusbildung ist noch nicht bekannt.

### B. Physiologisches.

Dieser Pilz wächst üppig auf Reis, Bohnen, Kartoffeln, Brot, Kojigelatine und Agar, sowie in Bouillonpeptongelatine. Auch auf der Oberfläche der flüssigen Nährböden bildet er die charakteristische Decke.

Besonders wenn die Kultur auf Pfeffer's Lösung, Bohnen u.a., bei  $23-25^{\circ}\text{C}$  angestellt wird, erzeugt diese Art binnen 20 Tagen leicht grosse Mengen Sklerotien auf der Myceldecke, mit Konidienträgern; aber bei Zimmer- und höherer Temperatur wird diese Bildung vermindert. Das Wachstum des Pilzes zeigt keinen Unterschied in einer Lösung von Glukose, Maltose, Saccharose, Stärkekleister, ist aber spärlich in Laktose. Bei den stickstoffhaltigen Nährlösungen begünstigen Ammoniumsalze, Aminosäuren, Pepton, Eiweiss und Säureamide das Wachstum im Grade ihrer Reihenfolge.

Die optimale Temperatur für das Wachstum des Pilzes ist  $23-27^{\circ}\text{C}$ , doch kann der Pilz auch bei  $40^{\circ}\text{C}$  noch mässig gedeihen.

Das Verflüssigungsvermögen des Pilzes für Gelatine wurde mit parallelen Kulturen von *Aspergillus Oryzae*, *Aspergillus albus*, *Aspergillus gymnosardae* verfolgt<sup>1</sup>. Der Pilz schmilzt binnen 20 Tagen bereits die Hälfte der Gelatine, indes *Asp. gymnosardae* und *Asp. albus* ein Drittel, *Asp. Oryzae* ein Sechstel verflüssigt.

Die Kraft des proteolytischen Enzyms<sup>2</sup> dieses Pilzes ist, wie oben bezeichnet, ausserordentlich kräftig; es verflüssigt nach kurzer Zeit koagulierte Eiweissstoffe, Fibrin, Gelatine bei 40° C, und zeigt dabei deutliche Biuretreaktion. Neutraler Zustand ist der Reaktion am günstigsten, im alkalischen verzögert sie sich und saurer ist ihr sehr nachteilig; ein Teil des Enzyms löst 100 Teile der 10 proz. neutralen Gelatine in 40 Minuten bei 40° C auf, aber unter derselben Bedingung nimmt es eine Stunde dieselben Teile der 2 Proz. Natriumkarbonat enthaltenden Gelatine zu verflüssigen, und ferner braucht es 1½ Stunde 0,06 Proz. Salzsäure enthaltende Gelatine zu lösen. Die Enzymlösung verzuckert Stärkekleister, invertiert die Saccharose, hydrolysiert auch die Maltose, das Inulin und die Mannane. Die Guajaktinktur bei Anwesenheit von Wasserstoffsuperoxyd wird durch das Enzym blau gefärbt, auch zersetzt es Wasserstoffperoxyd unter Sauerstoffentwicklung. Ferner hydrolysiert ein wässriger Auszug dieses Pilzes Monobutyryn<sup>3</sup>. Diese Versuche zeigen uns, dass der Pilz Amylase, Invertase, Glykase, Inulase, Seminase, Peroxydase, Katalase und Lipase ausscheiden kann.

Der Farbstoff wird durch Alkohol extrahiert.

Eine wichtige Tatsache ist, dass diese Art aus älterer Kultur auf Koji-gelatine Calciumoxalat-Krystalle erzeugt, und reichliche Mengen von saurem Ammoniumoxalat auf gedämpften Bohnen.

1. Strichkultur auf Koji-gelatine (15%) bei 8–15°C.

2. Enzympräparat wurde in üblicher Weise erzeugt.

3. Comptes rend. de l'Ac. 1897, Bd. 124 S. 370.

## C. Affinität.

Dieser Pilz steht in vielen Beziehungen *Aspergillus ochraceus* Wilhelm<sup>4</sup> und *Aspergillus auricomus* Gueguen<sup>5</sup> nahe, jedoch stimmt nach Wehmer<sup>6</sup> jedenfalls *Asp. auricomus* mit *Asp. ochraceus* überein.

Nach den Beschreibungen des *Asp. ochraceus* unterscheidet sich *Asp. melleus* dadurch, dass der Konidienträger von *Asp. ochraceus* stattlich und 2-3 mm. hoch ist (selbst 4-10 mm., aber nach Schröter<sup>7</sup> bis 1 mm.), bei *Asp. melleus* ist derselbe dagegen niedrig. Auch die Konidien des *Asp. ochraceus* sind 3,5 - 5  $\mu$ , aber die des *Asp. melleus* 2,5 - 4  $\mu$ . Ueberdies ist die Farbe des Konidienrasens bei *Asp. melleus* im Anfang bernsteinartig, später wird er weisslich braungelb, dagegen hat *Asp. ochraceus* eine Farbe von ockergelb bis braungelb. Auch bei direkten Kulturen des *Asp. ochraceus* Willh. (Went) sowie *Asp. ochraceus* Willh. (Tiraboschi), die mir neulich Herr Nakazawa übersandte, sahen wir, dass ein deutlicher Unterschied zwischen *Asp. ochraceus* und *Asp. melleus* vorhanden ist. Im direkten Vergleich zeigen beide Arten nicht nur obenerwähnte Merkmale, sondern *Asp. ochraceus* unterscheidet sich von unserer Art noch durch folgende Tatsache: der Köpfchendurchmesser des *Asp. ochraceus* ist kleiner als der des *Asp. melleus*, und die Konidienketten von *Asp. ochraceus* sind stets kürzer als die unserer Art.

## D. Diagnose.

Hyphen farblos, hell, septiert, auf Flüssigkeiten zu dichten Decken verflochten. Reife Konidienrasen bernsteinartig, später weisslich braun-

4. Wilhelm, K. Beiträge zur Kenntnis der Pilzgattung *Aspergillus* 1877.

5. Bull. Soc. Mycol. de France T. XV. 1899 p. 171.

6. Centrabl. f. Bakt. II Abt. 1907 Bd. XVIII S.392.

7. Wehmer. Die Pilzgattung *Aspergillus* 1901 S.115.

gelb mit zahlreichen, ziemlich stattlichen Konidienträgern; Stiel farblos, gerade oder gebogen, dünnwandig, Wand glatt oder warzig. Blase kugelig, seltener keulig, nicht scharf abgesetzt und allseitig von dicht gedrängt stehenden, verzweigten, radial ausstrahlenden Sterigmen besetzt. Primäre Sterigmen keulig, sekundäre kegelförmig, in der Regel zu 3-4. Sekundäre Sterigmen oft zu langer Strecke auswachsend. Konidienketten verhältnismässig nicht kurz. Reife Konidien meist kugelig, selten ellipsoidisch, stets glattwandig, kleinsporig. Sklerotien reichlich gefunden, ohne Askusbildung. Optimale Wachstumstemperatur 23-25° C.

Vorkommen: Auf getrocknetem Tunfisch (Japan) spontan auftretend. Gedeiht gut auf Bohnen, Brot, Gelatine, Fisch etc. Gebildet werden Amylase, Invertase, Inulase, Glykase, Seminase, Peroxidase, Katalase, Lipase, Protease.

#### GROESSENVERHAELTNISSE.

Hyphendurchmesser	... ..	2-5 $\mu$
Konidienträger	... ..	0,7-1 mm.
Stieldicke	... ..	7-25 $\mu$
Stielwanddicke	... ..	0,5-0,7 $\mu$
Köpfchendurchmesser	... ..	50-250 $\mu$
Blasendurchmesser	... ..	20-50 $\mu$
Primäre Sterigmen	... ..	10-22 $\mu \times 2,5-4 \mu$
Sekundäre Sterigmen	... ..	10-16 $\mu$ (selten bis 30 $\mu \times 1-2 \mu$ )
Konidiendurchmesser	... ..	2,5-4 $\mu$
Sklerotiendurchmesser...	... ..	0,3-0,7 mm.

## II. *Aspergillus gymnosardae* nov. spec. (Hierzu Taf. XVIII, Fig. 1-7d).

Dieser Pilz ist als „Awokabi“ bekannt und im praktischen Gebrauch sehr wichtig zur Fabrikation des „Katsuobushi“. Er tritt jedoch nicht

8. Awokabi bedeutet grüner Schimmelpilz, und gewöhnlich nennen wir *Penicillium glaucum* so, aber bei Katsuobushi ist es nicht *Penicillium glaucum*, welches fast gar nicht in Katsuobushi auftritt.

unter künstlicher Kultur auf, sondern spontan auf dem Fleische während der Einlagerung von „Katsuobushi“, wenn die Feuchtigkeit und die Temperatur in dem Fasse mässig sind. Ueber den praktischen Wert der Verwendung der kultivierten Sporen dieses Pilzes bei der Bereitung von „Katsuobushi“ werde ich mich ein andermal aussprechen. Ich weiss die Ursache noch nicht genau, warum diese Art am besten für die Fabrikation des „Katsuobushi“ ist, warum die Qualität der mit dieser Art bedeckten Proben fein und vorzüglich ist, während die mit *Asp. melleus* bedeckte Ware als gering gilt.

Nach der Farbe der Konidienrasen machen die grünen Arten das Gros der Gattung aus, aber die Beschreibungen für die grössere Reihe dieser Arten sind unvollständig. In folgendem werde ich nur diesen Pilz mit den kenntlich beschriebenen ähnlichen Arten nach Wehmer<sup>9</sup> vergleichen.

#### A. Morphologisches.

Auf den verschiedenen zur Kultur benutzten Substraten bildet dieser Pilz einen dicken, anfangs weissen, bald gelblich grünen, seltener laubgrünen Konidienrasen. Bei älteren Kulturen geht die Färbung schliesslich von unansehnlichem Grün bis ins schmutzig Dunkelbraune über. Gelbliche Töne an den auf verschiedenen Substraten gezogenen Decken des *Asp. flavus* treten immer tiefer auf als bei diesem Pilz. Die Konidienträger des Pilzes sind stattlich, 1-2,5 mm. hoch, (beim *Asp. flavus* 0,5-0,7 mm.) und meist einfach, seltener verzweigt, aber die älteren Träger sind mit vielen Querwänden versehen. Die Wand des Konidienträgers ist 1-2  $\mu$  dick, glatt oder auch rauhwarzig. Das Ende des Trägers quillt zu einer kugeligen oder keulenförmigen Blase (20-40  $\mu$  im Durchmesser), die in der Regel nicht scharf von dem Stiel abgesetzt ist.

9. Wehmer. Die Pilzgattung *Aspergillus* 1901 S. 61.

Lafer Handbuch der Tech. Mykologie Bd. IV S. 202.

Die Sterigmen sind radial von den Seiten der Blase ausstrahlend, oder bei kleineren Trägern mehr auf die Kuppe beschränkt und aufwärts gerichtet (bei *Asp. pseudoflavus* Saito<sup>10</sup> nur radial ausstrahlend). Sie sind farblos, meist einfach, doch auch verzweigt wie beim *Asp. candidus*, *Asp. Ostianus*, *Asp. pseudoflavus*. Im Falle der Verzweigung der Sterigmen sind die primären Sterigmen oben breit-keulig und die sekundären zart, schlank und zu drei oder vier auftretend (bei *Asp. pseudoflavus* zu zweien auftretend). Die einfachen Sterigmen haben eine Länge von 10-25  $\mu$  und eine Breite von 5-6  $\mu$  (in den verzweigten Sterigmen: primäre Sterigmen 10-20  $\mu \times 5-6 \mu$ , sekundäre 10  $\mu \times 2-3 \mu$ ).

Die Länge der Sterigmen überschreitet den Blasenradius und ist oft gleich dem Blasendurchmesser; der Pilz gehört also zu der „Langstrahlen“; diese Merkmale ermöglichen eine Unterscheidung von anderen ähnlich gefärbten Arten (in diesem Punkte unterscheidet er sich von *Asp. glaucus*, *Asp. Oryzae*, *Asp. flavus*, *Asp. pseudoflavus*). Der Inhalt der älteren, von den Sterigmen befreiten Blase erscheint oft den Konidien gleich gefärbt, wodurch wieder ein Unterschied gegen *Asp. Oryzae*, *Asp. flavus* und *Asp. pseudoflavus* gegeben ist. Die Konidien treten in leicht und bald zerfallenden langen Ketten auf; die Länge der Konidienketten dieses Pilzes stimmt mit *Asp. flavus* und *Asp. Oryzae* überein. Sie sind gross, allgemein kugelig, seltner schwach ellipsoidisch, meist warzig oder glatt und gelbgrünlich gefärbt. Nach der Konidiengrösse beurteilt, gehört diese Art zu den „grosssporigen“; die Grösse dieser Organe ist 4-6  $\mu$ , hierin steht er weniger hinter *Asp. Oryzae*, *Asp. flavus*, *Asp. pseudoflavus* zurück. Missbildungen der Konidenträger sind nicht selten.

Das Mycel der Art besteht aus zarten, septierten, farblosen Hyphen von 2-5  $\mu$  Durchmesser, und die älteren Hyphen werden öfter gelbbraun gefärbt wie bei *Asp. glaucus* und *Asp. varians*.

Sklerotien und Peritheecien sind noch unbekannt.

## B. Physiologisches.

Der Pilz gedeiht auf sehr verschiedenen Substanzen flüssigen wie festen Charakters; auf Kojidekoktlösung, Bierwürze, Reis, Brot, ist die Art leicht zu ziehen. Doch bevorzugt der Pilz Zuckerlösung ganz gleich ob mit Eiweiß oder mit anorganischen Stickstoffverbindungen neben Kaliumphosphat und Magnesiumsulfat. Die Farbe der Konidienrasen ist löslich in Alkohol, unlöslich in Wasser. Auf den Kulturen in Würze und Kojidekoktlösung bildet dieser Pilz Gasblasen. Auch liebt er hohe Temperaturen, ein Wachstumsmaximum liegt  $41^{\circ}\text{C}$ , aber er kommt mehr bei Blutwärme zur Entwicklung, bei Zimmertemperatur ist er ein wenig schwerfällig. Die Art bildet Amylase, Invertase, Glykase, Peroxydase, Katalase, Lipase und Protease.

## DIMENSIONEN.

Hyphendurchmesser	... ..	2-5 $\mu$
Konidienträger	... ..	1-2,5 mm.
Stiel Dicke	... ..	10-20 $\mu$
Stielwanddicke	... ..	0,8-1,2 $\mu$
Köpfchendurchmesser	... ..	100-500 $\mu$
Blasendurchmesser...	... ..	20-49 $\mu$
Einfache Sterigmen	... ..	12-25 $\mu \times 5-6 \mu$
Primäre Sterigmen	... ..	10-20 $\mu \times 5-6 \mu$
Sekundäre Sterigmen	... ..	10 $\mu \times 2-3 \mu$
Konidien ..	... ..	4-6 $\mu$



## TAFELERKLÄRUNG.

## Tafel XVII.

- Fig. 1-7 *Aspergillus melleus* nov. spec.  
 Fig. 1. (Vergr. 15) Ein Stück der Myceldecke mit den aufliegenden Sklerotien neben Konidienträgern (halb schematisch).  
 Fig. 2. (Vergr. 400) Konidienträger.  
 Fig. 3. (Vergr. 400) Entwicklungsstadien der Konidienträger.  
 Fig. 4. (Vergr. 560) Sterigmen.  
 Fig. 5. (Vergr. 700) Konidien.  
 Fig. 6. (Vergr. 400) Anormale Gestalten der Konidienträger.  
 Fig. 7. (Vergr. 125) Köpfchen mit langen Sterigmen.

## Tafel XVIII.

- Fig. 1-7. *Aspergillus gymnosardae* nov. spec.  
 Fig. 1. (Vergr. 52) Habitusbild des Köpfchens.  
 Fig. 2-4. (Vergr. 700) Konidienträger.  
 Fig. 2. Exemplar mit keuliger Blase und kuppelständigen Sterigmen.  
 Fig. 3. Wohlerhaltenes, gut entwickeltes Exemplar.  
 Fig. 4. (a-c) Zwergige Konidienträger.  
 Fig. 5 (Vergr. 700) a) einfache Sterigmen. b) verzweigte Sterigmen.  
 Fig. 6. (Vergr. 700) Konidien.  
 Fig. 7a-d (Vergr. 700) Abnormal geformte Konidienträger.
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1



3



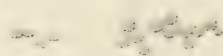
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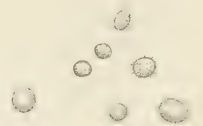
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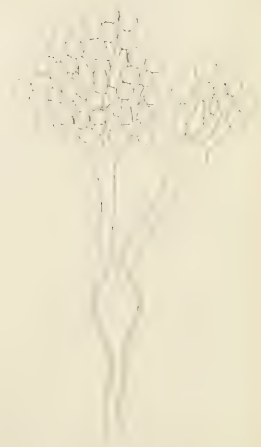
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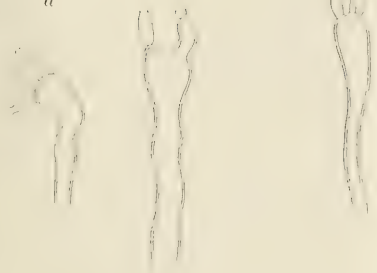


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b

c

a





# Influence of Rice Bran upon the Manurial Value of Phosphoric Acid Contained in Oil Cakes.

BY

Y. Kida.

In Japan various vegetable manures are most widely used by farmers, of which oil cakes from an important item. Oil cakes contain generally more nitrogen than phosphoric acid and they are treated rather as nitrogenous manure. Their effect of nitrogen has been studied already by many authors not only in our college but also in many of our prefectural experimental stations, but on the manurial value of the phosphoric acid contained in vegetable manures only little is known.

M. Nagaoka<sup>1</sup> concluded from his rice experiment that the manurial value of vegetable phosphoric acid was inferior to animal phosphoric acid, as the following table shows:

		Relative manurial value
Control-Double superphosphate ... ..		100
Animal manures	Shime-Kasu (Sardine) ... ..	85
	Shime-Kasu (Herring) ... ..	82
	Arakasu (Fish bone) ... ..	97
	Steamed bone meal ... ..	87
	Average ... ..	88

1. Bull. College of Agric., Tokyo, Vol. IV.

Vegetable manures	Rice bran ... ..	42
	Rape seed cake ... ..	30
	Sesame cake ... ..	38
	Soy bean cake ... ..	28
	Average ... ..	35

So it seems quite desirable to enhance the manurial value of phosphoric acid in vegetable manures; especially in oil cakes, as they are used very largely.

In recent times, various organic phosphoric compounds have been found in plants; besides nuclein and lecithin also phytin has been found widely distributed in the vegetable kingdom.

According to Tsuda's<sup>1</sup> analysis, the phosphoric acids contained in oil cakes are chiefly in three forms: lecithin, nuclein and phytin, of which the last contains the largest proportion of phosphoric acid, as follows:

IN 100 PARTS OF DRY MATTER

	Total $P_2O_5$	$P_2O_5$ in Lecithin	$P_2O_5$ in Nuclein	$P_2O_5$ in Phytin
Soy bean cake ... ..	1.311	0.114	0.236	0.640
Rape seed cake ... ..	2.251	0.091	0.204	0.873

Prof. K. Aso and T. Yosida concluded from their experiments that the manurial value of lecithin is much higher than that of nuclein and phytin, and the inferior value of vegetable phosphoric acid as compared with animal is accordingly well recognised.

This brief recapitulation shows the desirability of increasing the

1. Journal of the College of Agric. Vol. I., No. 2. P. 167.

2. The same Journal Vol. I., No. 2. P. 152.

availability of phytin as a first step towards a greater utilization of vegetable phosphoric acid for manurial purposes. Prof. U. Suzuki and K. Yoshimura<sup>3</sup> lately found an enzyme called phytase which splits phytin with the production of a soluble inorganic phosphoric compound and inosit, and which is widely distributed in plants.

As oil cakes are exposed to a high temperature to facilitate the separation of oil, either by streaming or by some other method, the activity of the contained phytase seems to be greatly reduced, and it is therefore of some interest to investigate the manuring value of the phosphoric acid of oil cakes can be enhanced by mixing rice bran which is known to contain much active phytin splitting enzyme. It was with this object in view that the following experiments were made under the direction of Prof. K. Asō.

#### **I. Experiment to investigate whether phytase is still active in Rape seed cake and Soy bean cake.**

A.—Rape seed cake, soy bean cake and rice bran purified of fatty matters with petroleum ether for 24 hours, were used in this experiment. Three Erlenmeyer's flasks were filled with 10 grams of these fine substances and 200 c.c. of distilled water, well stoppered with cotton and then subjected to the following treatments:

Flask a.—Left at room temperature.

„ b.—2 c.c. chloroform added, kept in the incubator at 30° C.

„ c.—Heated for one hour in Koch's sterilising kettle and then left at room temperature.

After seven days, the contents of each flask were filtered with dried filters and the filtrates were tested for inorganic soluble phosphoric acid with molybdic method, with the following results:

3. Bull. College of Agric. Tokyo Vol. VII., No. 4.

Flasks		P <sub>2</sub> O <sub>5</sub> found	
		in one flask (g)	in % of total P <sub>2</sub> O <sub>5</sub>
Rape seed cake (2.115% P <sub>2</sub> O <sub>5</sub> )	a	0.08672	41.90
	b	0.08848	41.83
	c	0.06272	29.65
Soy bean cake (1.208% P <sub>2</sub> O <sub>5</sub> )	a	0.07904	65.43
	b	0.07856	65.03
	c	0.06683	55.32
Rice bran (3.123% P <sub>2</sub> O <sub>5</sub> )	a	0.12292	39.36
	b	0.19588	62.72
	c	0.06276	20.09

This shows that in neither bean cake nor rape seed cake, the quantity of phosphoric acid underwent any great change with the different treatments, while in the case of rice bran the change was conspicuous.

Taking the average of b and c we get:

	P <sub>2</sub> O <sub>5</sub> (b-c)	
	(g)	in % of total P <sub>2</sub> O <sub>5</sub>
Rape seed cake ... ..	0.02516	12.15
Soy bean cake ... ..	0.01173	9.71
Rice bran ... ..	0.13312	42.62

These figures show that the activity of phytase in both rape seed and soy bean cakes is much affected when compared with that of rice bran, as was to be expected. As in (b) the action of microbes was excluded by chloroform, which has no influence upon phytase, while in (c) both these agents were inactivated, the difference of (b-c) will represent the activity of phytase.



B.—A similar experiment as in A was repeated and a very different result<sup>1</sup> was obtained. The experiment was carried out with only slight modifications in the treatment of the mixtures, as given below:

Flask a.—3 c.c. of chloroform added, then left at room temperature.

„ b.—3 c.c. of chloroform added, then left in the incubator at 35°—40° C.

„ d.—Heated for one hour in Koch's sterilising kettle, 3 c.c. of chloroform then added and left at the room temperature.

After standing for seven days, the contents of each flask was filtered and the soluble inorganic phosphoric acid was determined with the following results:

Flasks		P <sub>2</sub> O <sub>5</sub> found	
		in one flask (g)	in % of total P <sub>2</sub> O <sub>5</sub>
Rape seed cake (2.366% P <sub>2</sub> O <sub>5</sub> )	a	0.11324	47.86
	b	0.10968	46.36
	c	0.12216	51.63
	d	0.09104	38.48
Soy bean cake (1.339% P <sub>2</sub> O <sub>5</sub> )	a	0.09384	70.08
	b	0.07904	59.
	c	0.08544	63.81
	d	0.06632	49.54
Rice bran (4.286% P <sub>2</sub> O <sub>5</sub> )	a	0.20148	47.01
	b	0.21428	49.99
	c	0.28208	65.81
	d	0.05816	13.57

1. Substances used in all the following experiments were of just the same quality as in this experiment.

These figures show also that under these treatments the quantity of soluble phosphoric acids in the cakes is not conspicuously different from that of the rice bran, the difference between (c) and (d) in particular being very slight, but the activity of phytase was much more noticeable in rice bran than in the pressed cakes experimented on.

The average of (c) and (d) gives the following results:

	$P_2O_5$ (c-d)	
	(g)	in % of total $P_2O_5$
Rape seed cake ... ..	0.03112	14.27
Soy bean cake ... ..	0.01912	13.15
Rice bran ... ..	0.22392	52.25

It follows that these quantities of phosphoric acid were produced from phytin by the action of the phytase contained in these vegetable substances.

As the flasks were exposed to a high temperature, the separation of soluble phosphoric compounds must have been more or less accelerated by the heat, though the enzyme does not exert—accordingly the amount of  $P_2O_5$  in (c) seems in each case to be too large. The following experiment was carried out with the view of obtaining further light on the subject.

C.—The experiment was done under exactly the same conditions as the foregoing with the only difference that the flasks of a, b and c were also heated for one hour in Koch's sterilising kettle (as with d) just before being filtered after the various treatments for seven days. The results were as follows:

Flasks		P <sub>2</sub> O <sub>5</sub> found	
		in one flask (g)	in % of total P <sub>2</sub> O <sub>5</sub>
Rape seed cake ... ..	a	0.11500	48.61
	b	0.12140	51.31
	c	0.12396	52.39
	d	0.09108	38.50
Soy bean cake... ..	a	0.08160	60.94
	b	0.07904	59.03
	c	0.08626	64.38
	d	0.06555	48.96
Rice bran .. ...	a	0.29864	69.68
	b	0.26040	60.76
	c	0.39684	92.56
	d	0.12600	29.37

Keeping our attention to the essential factors concerned in the production of soluble phosphoric acid we note that (a) was allow free play to the actions of microbes, phytase and of heat, (b) to those of phytase and heat (c) to that of phytase in its beneficial condition and of heat, at last (d) to that of heat only, so that the action of phytase could be clearly brought out by comparing (c), as follows:

	P <sub>2</sub> O <sub>5</sub> (c-d)	
	(g)	in % of total P <sub>2</sub> O <sub>5</sub>
Rape seed cake ... ..	0.03288	13.89
Soy bean cake... ..	0.02065	15.42
Rice bran... ..	0.27084	63.19

These figures lead me to conclude that phytase exists in these cakes in an inactive or imperfectly active state, while in rice bran it exists in larger quantities or in a more active state, and there remains much phytin to be acted upon both in rape seed cake and soy bean cake. This led me to think that it would be interesting to investigate whether the phosphoric acid in the phytin of the cakes can be transformed into soluble inorganic form by mixing rice bran with them, as the active phytase contained in the latter seems to act well also on the phytin contained in the pressed cakes, and further whether the manurial value of the phosphoric acid of the pressed cakes, chiefly produced from phytin can be enhanced in this way.

## II. Experiments to investigate the behavior of rice bran towards the phosphoric compound contained in pressed cakes.

A.—In the following experiments the pressed cakes and rice bran used were treated with petroleum ether and freed from fatty matter.

- (i) Three Erlenmeyer's flasks were used for this experiment. One flask (a) was filled with 10 grams of the pressed cake and 200 c.c. of distilled water, well stoppered and heated in Koeh's sterilising kettle for one hour, and after cooling for a short time 2 grams of rice bran were added. In (b) were put 10 grams of the pressed cake and 200 c.c. of distilled water and heated as in (a); in (c) 2 grams of rice bran and 200 c.c. of distilled water and stoppered with cotton plug. (a) and (c) were then kept at 35°-40° C, and (b) left at the room temperature. After seven days, filtered with dried filter, and the filtrate from each flask analysed for soluble inorganic phosphoric acid with molybdcie method. To all the flasks were added previously 2 c.c. of chloroform.

The analytical results were:—

Flasks	Remarks	P <sub>2</sub> O <sub>5</sub> found	
		in one flask (g)	in % of total P <sub>2</sub> O <sub>5</sub>
a	10g heated bean cake +2g rice bran	..... 0.17648	83.35
b	10g heated bean cake	0.06708	} 0.14436 65.73
c	2g rice bran.	0.07728	
a-(b+c)		..... 0.03212	14.62
a	10g heated rape seed cake+2g rice bran	..... 0.21016	65.20
b	10g heated rapeseed cake	0.08416	} 0.16144 50.08
c *	2g rice bran	0.07728	
a-(b+c)		..... 0.04872	15.12

We see that the difference a-(b+c) amounts to 20.59% of the total phosphoric acid contained in the rape seed cake and to 23.99% of that in soy bean cake. These quantities of P<sub>2</sub>O<sub>5</sub> must have been derived from the phytin of the pressed cakes, and accordingly we may conclude that the rice bran acts favorably on the phytin of the pressed cakes, as was to be expected.

- (ii) Another experiment on the same basis was carried out with the only difference that the flasks were not exposed to heat. After leaving for seven days at 35-40° C the filtrate from each flask was analysed with the following results:—

\* The result given is the mean of two similar flasks. This is the case for the corresponding item in all the following tables.

Flasks	Remarks	P <sub>2</sub> O <sub>5</sub> found	
		in one flask (g)	in % of total P <sub>2</sub> O <sub>5</sub>
a	10g bean cake +2g rice bran	0.09688	89.65
b	10g bean cake	0.09488	78.63
c	2g rice bran	0.07780	
a-(b+c)		0.02420	11.02
a	10g rape seed cake +2g rice bran	0.25636	79.60
b	10g rape seed cake	0.11704	60.75
c	2g rice bran	0.07780	
a-(b+c)		0.06172	18.85

The results were quite in accordance with the expectation, the soluble phosphoric acid in (a) was increased to such an extent that it exceeded the sum of (b) and (c); the difference  $a-(b+c)$  amounted to 18.07% of the total phosphoric acid contained in the bean cake and to 16.06% of that in rape seed cake. This was mainly due to the action of phytase contained in rice bran.

B. In the following experiments all the manures were used in a finely pulverized form without freeing them from fatty matters.

- (i) The same experiment as (i) of A was repeated with the modification that the quantity of rice bran was increased to 5 grams in each case. The flasks (a) and (c) were kept at 30° C, while (b) was left at the room temperature. After seven days' standing, their filtrate was examined for soluble phosphoric acid.

Flasks	Remarks	P <sub>2</sub> O <sub>5</sub> found	
		in one flask (g)	in % of total P <sub>2</sub> O <sub>5</sub>
a	10g heated bean cake +5g rice bran	0.21268	61.68
b	10g heated bean cake	0.05304	48.19
c	5g rice bran	0.11476	
a-(b+c)		0.04488	12.89
a	10g heated rape seed cake +5g rice bran	0.20964	46.49
b	10g heated rape seed cake	0.06376	39.59
c	5g rice bran	0.11476	
a-(b+c)		0.03112	6.90

The difference a-(b+c) amounts to 13.15% of the total phosphoric acid contained in rape seed cake and to 33.52% of that in soy bean cake.

From the above table it is seen that the rice bran may act on pressed cakes in their raw condition at 30° C so as to increase their manurial value.

- (ii) A similar experiment as (ii) of A was carried out, but in this case 10 grams of rice bran were used with (a) and (c) each and the results were as follows:—

Flasks	Remarks	P <sub>2</sub> O <sub>5</sub> found	
		in one flask (g)	in % of total P <sub>2</sub> O <sub>5</sub>
a	10g bean cake +10g rice bran	0.26320	46.79
b	10g bean cake	0.08672	44.16
c	10g rice bran	0.16168	
a-(b+c)		0.01480	2.63

a	10g rape seed cake +10g rice bran	..... 0.31420	47.23	
b	1 g rape seed cake	0.11860	} 0.28028	42.13
c	10g rice bran	0.16168		
a-(b+c)		..... 0.03392		5.10

The difference  $a-(b+c)$  is small in bean cake and rape seed cake, but compared with the total amount of phosphoric acid in each cake it is 14.33% in rape seed cake and 11.05% in bean cake.

(iii) One more experiment was made this time reducing the rice bran to half the quantity used in the foregoing.

Flasks	Remarks	$P_2O_5$ found		
		in one flask (g)	in % of total $P_2O_5$	
a	10g bean cake +5g rice bran	..... 0.21116	60.64	
b	10g bean cake	0.08672	} 0.20132	57.82
c	5g rice bran	0.11460		
a-(b+c)		..... 0.00984		2.82
a	10g rape cake +5g rice bran	..... 2.29228		64.92
b	10g rape seed cake	0.11860	} 0.23320	51.72
c	5g rice bran	0.11460		
a-(b+c)		..... 0.05908		13.10

Rice bran brought about in this case also the decomposition of the phosphoric compound contained in the pressed cakes. 22.94% of the total phosphoric acid contained in the rape seed cake were changed by the action of rice bran into a soluble form; and in soy bean cake the corresponding figure was 7.35%.



SUMMARY OF RESULTS.

I. The existence of phytase both in rape seed cake and soy bean cake is certain, but its action in each case is very small.

II. Rice bran acts in such a way as to transform the organic phosphoric compounds of the latter to simple inorganic soluble ones, when mixed under suitable conditions; thus enhancing the manurial value of the phosphoric acid of the pressed cake.

III. The above result can be obtained not only in the pressed cakes freed from fatty matters, but also in the raw state.

† All the experiments were made with fresh materials.

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# Ueber Oryzanin, ein Bestandteil der Reiskleie und seine physiologische Bedeutung.

VON

U. Suzuki, T. Shimamura und S. Otake.

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Mit Tafeln XIX-XXVI.

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## I. Einleitung.

Im Jahre 1897 hat EIJMANN<sup>1</sup> zum ersten Male beobachtet, dass Hühner durch ausschliessliche Fütterung mit geschältem, sorgfältig von der Silberhaut befreitem Reis in kurzer Zeit den Appetit verlieren und unter starker Abmagerung zugrunde gehen. Er hat ferner darauf aufmerksam gemacht, dass diese Erscheinung mit der Beriberi-Krankheit des Menschen grosse Aehnlichkeit hat. Werden die Hühner mit ungeschältem Reis oder mit geschältem Reis und Kleie gefüttert, so bleiben sie nicht nur am Leben sondern selbst die Erkrankten werden bald damit geheilt.

Seine Beobachtung ist später von verschiedenen Autoren nachgeprüft und bestätigt worden. Ueber die Ursache derselben giebt es jedoch keine befriedigende Erklärung, so dass die Meinungen weit auseinander gehen.

Nach EIJMANN ist diese Erscheinung eine Vergiftung durch irgend einen Giftstoff im Stärkemehl von geschältem Reis, oder Gifte, welche

1. EIJMANN, Eine beriberiähnliche Krankheit der Hühner. Virchow's Archiv, Bd. 148, S. 523 (1897). —, Ein Versuch zur Bekämpfung der Beriberi. Virchow's Archiv, Bd. 149, S. 187 (1897). —, Archiv für Hygiene. 1906.

sich bei der Gärung von Stärkemehl in den Verdauungsorganen oder durch anomalen Stoffwechsel im Körper erzeugen. Nach MAURER<sup>1</sup> ist diese Krankheit eine Vergiftung durch Gärungsprodukte von Stärkemehl in den Verdauungsorganen, besonders des Oxalsäure. SAKAKI<sup>2</sup> glaubte, dass sie durch die Einwirkung der Giftstoffe, die durch Einwirkung der Bakterien auf geschältem Reis entstehen, verursacht werde. Dagegen schrieb MATSUSHITA<sup>3</sup> die Schuld dem Mangel an Eiweiss zu, SCHAUMANN<sup>4</sup> dem Mangel an organischen Phosphorverbindungen.

Wir wollen hier nicht in einzelne Details eingehen. Die Geschichte der Beobachtungen, Literatur usw. findet man in der „Mitteilung der Kakke-Studienkommission des japanischen Kriegsministeriums“ (1911).

Man kann nur mit Sicherheit sagen, dass die Reiskleie irgend einen Stoff enthält, welcher fähig ist, die erkrankten Tiere wieder zu heilen oder Erkrankung vorzubeugen. Was für ein Stoff ist das nun? Wir haben seit vier Jahren, teils gemeinsam mit Direktor Y. KOZAI und Dr. ANDO<sup>5</sup> und teils mit Dr. KITAO<sup>6</sup> und WATANABE u. a. auf diesem Gebiete gearbeitet. Nachdem wir die Beobachtung von EIJMANN nachgeprüft und bestätigt haben, gingen wir einen Schritt weiter, um den wirksamen Stoff der Kleie zu isolieren und die chemische Natur desselben genauer kennen zu lernen.

Wir stellten zunächst folgendes fest:—

1. Der aetherische Extrakt der Kleie hat keine Wirkung; die entfettete Kleie ist ebenso wirksam wie nicht entfettete Kleie.

1. MAURER, Archiv. f. Schiffs u. Tropenhyg. Bd. 13, II. S. u. 9, 1909.
2. SAKAKI, Untersuchungen über giftigen Reis. Tokio 1902.
3. MATSUSHITA, Ueber die Aetiologie der Kakke Krankheit. Zeits. f. Hygiene u. Bakteriologie, Bd. 2, S. 437, 1906 (japanisch).
4. SCHAUMANN, Archiv f. Schiffs- u. Tropenhygiene. Beiheft Bd. XII, 1908, u. Bd. XIII, 1909. Vergl. auch NOCHT, Ueber den gegenwärtigen Stand d. Beriberi-Frage. Ibidem. S. 15
5. KOZAI, ANDO, SUZUKI, und SHIMAMURA, Ueber die sog. „Beriberiähnliche Krankheit“ der Vögel. Special Report of the Agricultural Experiment Station Tokio 1910, August.
6. SUZUKI, SHIMAMURA, KITAO u. A., Journal of the Tokyo Chemical Society. Vol. 32, No. 1 (1911. Januar.), No. 2 (Februar 1911), No. 3 (April 1911), No. 9 (Sept. 1911), Vol. 33, No. 2 (Feb. 1912).

2. Wird die entfettete Kleie mit heissem Alkohol wiederholt extrahiert, so geht der wirksame Stoff in die alkoholische Lösung über und der Rückstand erweist sich vollständig wirkungslos.<sup>1</sup> Da der geschälte Reis sehr arm an anorganischen Bestandteilen, wie Phosphor, Eisen, Calcium, Magnesium, Kalium etc. ist, so haben wir zuerst geglaubt, dass die Tiere durch Mangel an Mineralstoffen leiden müssen, wenn sie ausschliesslich mit geschältem Reis gefüttert werden. Diese Annahme kann aber kaum richtig sein, weil der mit Aether und Alkohol extrahierte Rückstand der Kleie immer noch reich an Eiweiss, Stärke, Faser, Phytin, Salzen etc. ist. Ferner haben wir festgestellt, dass Kasein, Pepton, Eieralbumin, Lecithin, Phytin, anorganische Salze etc. keine Schutz- oder Heilwirkung gegen die Krankheit haben. Nach KAJIURA<sup>2</sup> hat das in Alkohol lösliche Eiweiss der Gerste keine spezifische Wirkung.

3. Der alkoholische Extrakt der Kleie stellt einen sauer reagierenden, dickbraunen Syrup dar, der sehr reich an Zucker, organischen Säuren, Lecithin, harzartigen Substanzen und anderen Verunreinigungen ist. Wird nun dieser alkoholische Extrakt in wenig Wasser gelöst, mit Schwefelsäure schwach angesäuert und mit Phosphorwolframsäure versetzt, so entsteht ein flockiger Niederschlag der die Hauptmenge des wirksamen Stoffes mitreisst, während Zucker, organische Säuren und andere Verunreinigungen meistens in der Mutterlauge zurückbleiben. Durch Zerlegung dieses Niederschlages durch Baryt erhält man einen schwachsauren, hellbraunen Syrup, welcher etwa 10 mal wirksamer als der alkoholische Extrakt ist. Für dieses Präparat haben wir den Namen „Roh-Oryzanin (I)“ gewählt.<sup>3</sup>

4. Wenn man das Roh-Oryzanin (I) in wenig Wasser löst und mit

1. Vergl. H. FRASERS, u. A. STAUTON, Studies from the Institute for Medical Research, Federated Manila States 1909; Y. TERUUCHI, Mitteilung der Kakke-Studienkommission des Japan. Kriegsministeriums (1911); Z. TSUZUKI. Ibidem.

2. KAJIURA u. O. ROSENHEIM, A Contribution to the Etiology of Beriberi. Jour. Hyg. (Cambridge), 10 (1910), No. 1. pp. 45—55.

3. Diese Beobachtung ist schon in The Journal of the Tokyo Chemical Society, Vol. 32, No. 1 (Jan. 1911) mitgeteilt.

Tannin versetzt, so wird ein Teil des Oryzanins mitgefällt. Nach Zerlegung dieses Tanninniederschlages durch Baryt und Entfernung des überschüssigen Baryts mittels Schwefelsäure erhält man einen hellbraunen Syrup [Roh-Oryzanin (II)], der nunmehr dreimal wirksamer als Roh-Oryzanin (I) ist.<sup>1</sup>

Ein ziemlich reines Präparat kann man auch aus alkoholischem Extrakt unmittelbar durch Tanninfällung erhalten.

Vor kurzem ist es uns gelungen aus Roh-Oryzanin (II) mittels Pikrinsäure den wirksamen Stoff Oryzanin in reinem Zustande zu isolieren.<sup>2</sup> Da die Ausbente des Pikrats sehr geringfügig ist, sind wir noch nicht imstande, die chemische Natur desselben aufzuklären. Wir hoffen aber, bald darüber Näheres mitteilen zu können.

5. Wird nun 0,005 bis 0,01 g des aus diesem Pikrate dargestellten Oryzanins einer durch ausschliessliche Reisfütterung erkrankten Taube per os gegeben oder subcutan eingespritzt, so wird das Tier in einigen Tagen geheilt; der Appetit kommt bald zurück und das Körpergewicht nimmt nach und nach zu. Man kann die Taube beliebig lange am Leben erhalten, wenn man dem geschälten Reis täglich 0,005 bis 0,01 g Oryzanin zugiebt. Ohne dies geht das Tier in 2 bis 3 Wochen zugrunde. Da eine Taube von ca. 300 g Körpergewicht täglich 25 bis 30 g Reis frisst, so macht das Oryzanin nur  $\frac{1}{2500}$  bis  $\frac{1}{5000}$  des Futtermittels aus. Es ist eine auffallende Tatsache, dass eine so kleine Menge des Oryzanins einen so grossen Einfluss auf die Ernährung des Tieres hat.

6. Es fragt sich nun, ob das Oryzanin bei anderen Tieren auch eine ebenso wichtige Rolle spielt, wie bei Tauben. Bei Hühnern, Mäusen und Hunden haben wir beobachtet, dass das Verhalten des Oryzanins genau dasselbe ist wie bei Tauben. Mäuse sterben gewöhnlich in 10 bis 15 Tagen wenn sie ausschliesslich mit geschältem Reis gefüttert werden. Sie bleiben aber längere Zeit gesund und normal, wenn man den alkoholischen Extrakt der Kleie oder das Roh-Oryzanin zugiebt.

1. Journal of the Tokyo Chemical Society, Vol. 32, No. 4. u. 9 (April u. Sept. 1911).

2. Ibidem. Vol. 33, No. 2 (Feb. 1912).

Wenn man Hunde mit gekochtem Reis und ausgekochtem Rückstand des Pferdefleisches füttert, so beobachtet man am Anfange keine Störung. Erst 2 bis 3 Wochen später geht der Appetit nach und nach zurück und nach 5 bis 7 Wochen gehen die Tiere unter starker Abmagerung zugrunde. Nur 3 bis 4 g alkoholischer Extakt der Kleie oder 0,3 bis 0,4 g Roh-Oryzanin (I) kann einen absterbenden Hund in ein paar Tagen heilen. Der Appetit kommt bald zurück und das Körpergewicht nimmt sehr rasch zu. Wird die Oryzaninzugabe eingestellt, so wird das Tier wieder krank. Mit einem ausgewachsenen Hunde haben wir in 7 Monaten 4 mal denselben Versuch wiederholt.

Da die Fette oder Salze keinen merkbaren Einfluss in diesen Fällen zeigen, so nehmen wir an, dass das Oryzanin einen für Erhaltung des tierischen Lebens unentbehrlichen Stoff bildet. Mit reinem Eiweiss, Fett, Kohlehydraten und Salzen konnten die Tiere nicht längere Zeit am Leben erhalten werden. Es fehlt noch Oryzanin dazu.

Um diese Annahme weiter zu stützen, haben wir Tauben und Mäuse mit einem Futtergemische gefüttert, welches aus einzelnen isolierten Nährstoffen zusammengestellt war. Zwei Tauben wurden mit Kartoffelstärke, Pepton, Lecithin, Phytin und Salzen gefüttert, zwei andere bekamen noch dazu, 0,03 g Roh-Oryzanin (I). Der Unterschied zwischen beiden Gruppen war auffallend. Die ersten zwei Tauben gingen in 10 bis 15 Tagen unter starker Abmagerung zugrunde, während die letztere nicht nur vollständig gesund blieben, sondern sogar bedeutend an Körpergewicht zugenommen haben. Anstatt Pepton haben wir auch Kasein, Eialbumin und Kleie-Eiweiss angewendet. (Das Kleie-Eiweiss wurde durch verdünntes Alkali aus der Kleie extrahiert und mit Essigsäure gefällt.) Die Ergebnisse waren auch genau dieselben. Ferner haben wir Tauben mit einem eiweissfreien Futtergemisch gefüttert. Die Tiere konnten natürlich nicht lange leben. In kurzer Zeit gingen sie zugrunde. Trotzdem lebten diejenigen, die Oryzanin bekamen, 3 mal länger als die, die kein Oryzanin erhielten. Die tägliche Abnahme des Körpergewichts bei den ersten war ungefähr  $\frac{1}{3}$  des der letzteren.



Welche Rolle das Oryzanin im Tierorganismus spielt, wissen wir gegenwärtig nicht. Es stellt nur fest, dass es zur Erhaltung des tierischen Lebens unentbehrlich ist, wenigstens für Tauben, Hühner, Mäuse und Hunde.

Es sei hier erwähnt, dass verschiedene Autoren schon mehrfach Versuche angestellt haben, um die Tiere längere Zeit, mit einem künstlichen Futtermische, aus einzelnen isolierten Nährstoffen am Leben zu halten. Die meisten Versuche erwiesen sich als erfolglos. Blos RÖHMANN<sup>1</sup> und OSBORNE<sup>2</sup> haben in neuerer Zeit etwas bessere Resultate bekommen. Obgleich es keinen Zweifel giebt, dass die Konstitution des Eiweisses, die Mengenverhältnisse und Verbindungsformen der Mineralbestandteile etc. einen grossen Einfluss auf das Gelingen der Versuche haben werden, darf man doch bei Tierversuchen nie das Oryzanin übersehen. Ohne Zweifel hätten RÖHMANN und OSBORNE noch bessere und befriedigendere Resultate gehabt, wenn sie in ihren Futtermischen das Oryzanin zugegeben hätten.

7. Da wir bis jetzt keine zuverlässige Methode ausgearbeitet haben, um das Oryzanin in verschiedenen Futtermitteln zu bestimmen, so blieb uns nichts übrig, als durch Tierversuche die annähernde Menge desselben zu ermitteln. Zu diesem Zwecke wurden die Tauben mit verschiedenen Futtermitteln mit geschältem Reis zusammen gegeben. In dieser Weise haben wir festgestellt, dass Weizen und Gerstenkleie, Bohnen, Hirse, Hafer, Gemüse etc. instande sind, die Tiere längere Zeit am Leben zu halten, ohne an Körpergewicht zu verlieren. Interessant ist dass die sorgfältig entkleiete Gerste, nach dem Kochen und Waschen mit Wasser, immer noch fähig war, die Tiere mehr als 100 Tage gesund zu halten. Auch gewöhnliches Weizenbrot erwies sich als wirksam.

In Miso, Sohyu, Bier und Sake haben wir kein Oryzanin nachgewiesen.

Ob der wirksame Stoff in verschiedenen Futtermitteln immer mit dem Oryzanin der Reiskleie identisch ist, oder ob es sich um eine Körperklasse handelt, können wir gegenwärtig nicht entscheiden.

1. RÖHMANN, Klin.-therap. Wochenschr. Nr. 40, 1902 und Allg. med. Zentralztg. 1908, Nr. 9.

2. OSBORNE, Science N. S., Vol. XXXIV, No. 882, pp. 722-732 (24. Nov. 1911).



Milch, Eier, Fisch und Pferdefleisch als solche, oder der alkoholische Extrakt derselben haben fast keine Wirkung auf Tauben gezeigt. Bei Hunden und Mäusen war das Verhalten etwas anders. Der alkoholische Extrakt des Pferdefleisches war für Hunde ebenso wirksam wie das Oryzanin. Für Mäuse war der letztere etwas ungünstiger, trotzdem konnten wir in einigen Fällen die Mäuse mehr als 50 Tage vollkommen gesund erhalten (bei Zugabe des alkoholischen Extrakts des Pferdefleisches mit geschältem Reis zusammen).

Der alkoholische Extrakt der Milch war auch fähig, Mäuse mehr als 50 Tage gesund zu halten, während der Rückstand derselben sich vollständig als unwirksam erwies.

Es soll deshalb unsere zukünftige Aufgabe sein, die wirksamen Stoffe aus verschiedenen Futtermitteln zu isolieren und ihre chemische Natur und physiologischen Funktionen zu studieren.

## II. Darstellung des Oryzanins.

300 g entfettete Reiskleie werden in einem Rundkolben mit 1 l. Aethylalkohol (85 bis 90%) in Verbindung mit Rückflusskühler 3 Stunden lang gekocht. Man filtriert heiss ab, kocht den Rückstand noch 1 Stunde mit  $\frac{1}{2}$  l. Alkohol und saugt wieder ab; diese Operation wird 4 mal wiederholt. Die gesamten alkoholischen Auszüge werden nun unter vermindertem Druck so lange eingedampft, bis der Alkohol vollständig ausgetrieben ist. Der zurückgebliebene dickbraune Syrup wird wiederholt mit Aether geschüttelt, um die Fette, organischen Säuren, Lecithine und andere Verunreinigungen zu entfernen, und weiter bei gelinder Wärme eingedampft. So erhält man einen ziemlich stark sauer reagierenden braunen Syrup, den wir der Einfachheit halber als „alkoholischen Extrakt“ bezeichnen. Die Ausbeute desselben beträgt ungefähr 30 g, d. h. ca. 10% des Ausgangsmaterials.<sup>1</sup>

1. Aus heissem alkoholischen Auszug scheidet sich beim Erkalten ein weisser, flockiger Niederschlag ab, der mit wenig warmem Alkohol gewaschen, in heissem Benzol gelöst und durch Zusatz von Alkohol gereinigt wird. Es bildet ein schuppenförmiges Pulver mit wachsähnlichem Glanz und schmilzt bei 84°C. Die Analyse gab C=80.44, H=13.33%. Die einfachste Formel wäre dann  $C_{27}H_{34}O$ .

Der alkoholische Extrakt wird nun mit Wasser auf 100 c.c. verdünnt und mit so viel Schwefelsäure angesäuert, bis sie ungefähr 3% der Flüssigkeit ausmacht, und mit einer 30% igen Phosphowolframsäurelösung versetzt. Es entsteht dabei ein brauner flockiger Niederschlag in reichlicher Menge. Man braucht etwa 20 bis 30 c.c. Phosphowolframsäurelösung dazu. Ein Ueberschuss des Reagens ist zu vermeiden. Nach einigen Stunden wird der Niederschlag abgesaugt, mit 3% iger Schwefelsäure einmal gewaschen. Man bringt den Niederschlag in einen Mörser, gibt etwas Wasser zu, und verreibt mit überschüssigem Barythydrat, bis der dicke Brei stark alkalisch reagiert (oder, man löst den Niederschlag in acetonhaltigem Wasser und gibt so viel Barythydrat zu, bis die Flüssigkeit stark alkalisch reagiert.) Nach einiger Zeit saugt man ab und behandelt den Rückstand noch 3 mal in derselben Weise. Das gesamte Filtrat wird nun durch Schwefelsäure sorgfältig von Baryt befreit und bei niedriger Temperatur unter vermindertem Druck eingedampft. Es bleibt dabei ein schwach sauer, hellbrauner Syrup zurück, der beim Trocknen über Schwefelsäure zu einer harzartigen Masse sich verwandelt. Wir haben für dieses Präparat den Namen „Roh-Oryzanin (I)“ vorgeschlagen. (Früher nannten wir es „Aberisäure“<sup>1</sup>. Da aber das reine Oryzanin keine saure Natur besitzt, so haben wir den Namen fallen lassen.) Die Ausbeute an Roh-Oryzanin (I) beträgt ca. 1,2 g aus 300 g Kleie, d. h. 0.4% des Ausgangsmaterials.

Wird nun 0,03 bis 0,04 g Roh-Oryzanin (I) in wenig Wasser gelöst, und einer durch ausschliessliche Reisfütterung erkrankten Taube per os eingegeben oder subcutan eingespritzt, so wird das Tier schon am nächsten Tage munter. Der Appetit kommt zurück, das Körpergewicht steigt, und nach 3 bis 4 Tagen bemerkt man keine Zeichen der Erkrankung mehr. Bekommt das Tier nur die halbe Dosis, so bleibt es längere Zeit am Leben, ohne jedoch vollständig geheilt zu werden. Das Körpergewicht steigt nicht auf. Eine grössere Menge Oryzanin hat keine schädliche Wirkung. Wir haben einmal 5g alkoholischen Extrakt einer Taube gegeben,

1. U. SUZUKI und T. SHIMAMURA, Journal of Tokyo Chemical Society, Vol. 32, No. 1 (1911).

ohne irgend eine Störung zu beobachten. Die Wirkung des Roh-Oryzanins (I) ist deshalb ungefähr 10 mal grösser als die des alkoholischen Extrakts selbst, d. h. 0,03 g des ersteren wirkt ebenso gut wie 0,3g des letzteren.

Das Filtrat von Phosphowolframsäureniederschlag war beinahe frei von Oryzanin. Wenn man das Filtrat mit so viel Barythydrat versetzt, bis es schwach alkalisch reagiert, so wird die Schwefelsäure sowie die Phosphowolframsäure vollständig gefällt. Wird nun der dabei entstandene Niederschlag abgesaugt und der Ueberschuss von Baryt durch Schwefelsäure sorgfältig entfernt, klar abfiltriert und bei niedriger Temperatur unter vermindertem Druck eingedampft, so bleibt ein hellbrauner Syrup zurück. Diese Präparat hat nun kein Schutz- oder Heilwirkung mehr auf erkrankte Tauben. Es scheint also, dass der Hauptanteil des Oryzanins durch Phosphowolframsäure gefällt, und was noch in Lösung geblieben war, durch weitere Behandlung beinahe verloren gegangen ist.

#### Reaktionen des Roh-Oryzanins (I).

Das in oben erwähnter Weise dargestellte Roh-Oryzanin (I) löst sich in Wasser und in verdünntem Alkohol sehr leicht. Die Lösung reagiert schwach sauer; gibt keine Biuretreaktion; mit MILLOX'schem Reagens erwärmt färbt sich die Lösung dunkelrot; aus konzentrierter Lösung entsteht sogar eine rotbraune Fällung. Phosphowolframsäure oder Phosphomolybdänsäure ruft in angesäuerter Lösung von Oryzanin eine flockige Fällung hervor; FEHLING'sche Lösung gibt bei gewöhnlicher Temperatur eine schmutzig-grüne Färbung, beim Erwärmen entsteht ein flockiger Niederschlag. Mit Natronkalk erhitzt, entwickelt sich Ammoniak. Werden einige Tropfen NESSLER'scher Reagens der Oryzaninlösung zugesetzt, so wird die Flüssigkeit bei gewöhnlicher Temperatur allmählich rotbraun und beim Erwärmen gibt sie einen dunkelbraunen Niederschlag. Beim Glühen hinterlässt das Roh-Oryzanin keine Asche.

Eine charakterische Reaktion für Roh-Oryzanin (I) ist jedoch die „Diazoreaktion“. Wird frisch bereitete p-Diazobenzolsulfonsäure in etwa

100 Teilen ganz verdünnter Natronlauge gelöst (die Lösung soll nur schwach gelb gefärbt sein), und werden einige Tropfen Roh-Oryzaninlösung zugefügt, so nimmt die Flüssigkeit sofort eine blutrote Färbung an und zugleich merkt man eine geringe Schaumentwicklung. Nach 5 bis 10 Minuten erreicht die Maximumintensität, die einige Tage unverändert bleibt.

Phosphomolybdänsäure gibt eine weisslichgrüne Färbung; durch Zusatz von Ammoniak wird der Niederschlag gelöst; die Flüssigkeit nimmt dabei eine tiefe indigoblaue Färbung an. Eine blaue Jod-Stärke-Lösung wird durch Zusatz von einiger Tropfen Oryzanin sofort entfärbt.

Eine konzentrierte wässrige Lösung des Oryzanins wird durch Bleiessig teilweise gefällt, durch Zusatz von Ammoniak wird die Fällung vollständiger. Queckilberekhlörld, -acetat und -nitrat oder Gerbsäure geben dabei eine unvollständige Fällung.

#### Spaltungsprodukte des Roh-Oryzanins (I).

Durch verdünnte Mineralsäuren oder Alkalien wird das Oryzanin leicht gespalten; die eigentümliche Wirkung geht dabei vollständig verloren. Emulsin wirkt auch allmählich auf Oryzanin ein. Ohne Zweifel ist das Oryzanin eine sehr labile Verbindung; daraus kann man die Beobachtung von EIJMANN, SHIGA<sup>1</sup> u.a., dass längere Zeit aufbewahrte oder verschimmelte Kleie keine Schutz- oder Heilwirkung mehr besitzt, leicht erklären.

Wenn man 1 g. Roh-Oryzanin (I) mit 100 c.c. 3% iger Salz- oder Schwefelsäure 2 Stunden erhitzt, so wird die klare Flüssigkeit allmählich trüb und auf der Oberfläche der Flüssigkeit scheidet sich eine harzartige Substanz ab. Filtriert man nun heiss ab und lässt das Filtrat einige Stunden stehen, so scheiden sich gelbbraune Krystalle in kleiner Menge aus; sie werden abgesaugt, mit wenig kaltem Wasser gewa-

1. SHIGA u. KUSAMA, Mitteilung d. Kakke-Studienkommission d. jap. Kriegsministeriums (1911).

sehen und aus heissem Alkohol umkrystallisiert. Aus alkoholischer Lösung erhält man zwei verschiedene Sorten Krystalle; sie lassen sich durch ungleiche Löslichkeit in Alkohol leicht von einander trennen. Sie werden vorläufig als  $\alpha$ - und  $\beta$ -Säure bezeichnet, (Taf. XIX, II u. III), die erstere ist in Alkohol viel schwerer löslich als die letztere. Sie werden nochmal für sich aus heissem Alkohol umkrystallisiert, mit wenig Alkohol und Aether gewaschen, im Vakuum bei  $100^\circ$  getrocknet und analysiert. (Aus alkoholischer Lösung scheiden sich die Krystalle durch Zusatz von wenig Salzsäure viel leichter aus.)

Analyse der  $\alpha$ -Säure.

1.	0.1129 g Subst. gaben	0.2206 g $\text{CO}_2$	0.0426 g $\text{H}_2\text{O}$
2.	0.0935 g „ „	0.1826 g $\text{CO}_2$	0.0373 g $\text{H}_2\text{O}$
3.	0.1514 g „ „	0.2960 g $\text{CO}_2$	0.0598 g $\text{H}_2\text{O}$
4.	0.0722 g „ „	4.4 c.c. N ( $18^\circ$ , 766 mm)	
5.	0.0963 g „ „	5.7 c.c. N ( $17^\circ$ , 764 mm)	
$\text{C}_{10}\text{H}_8\text{NO}_4$	Ber.	C 53.46	H 3.96
			N 6.93
	Gef.	53.29	4.19
		53.26	4.43
		53.32	4.39
			7.19
			—
			6.91

Analyse der  $\beta$ -Säure.

1.	0.1267 g Subst. gaben	0.2676 g $\text{CO}_2$	0.0419 g $\text{H}_2\text{O}$
2.	0.1244 g „ „	0.2640 g $\text{CO}_2$	0.0431 g $\text{H}_2\text{O}$
3.	0.1005 g „ „	0.2128 g $\text{CO}_2$	0.0354 g $\text{H}_2\text{O}$
4.	0.1509 g „ „	9.0 c.c. N ( $21^\circ$ 763 mm)	
$\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_9$	Ber.	C 58.25	H 3.88
			N 6.80
	Gef.	57.60	3.68
		57.88	3.85
		57.71	3.91
			6.82
			—
			—

Die beiden Säuren sind in kaltem Wasser schwer, in heissem Wasser etwas leichter löslich; die wässrige Lösung reagiert ziemlich stark sauer.

In Alkohol oder in Alkalien werden sie leicht gelöst und durch Zusatz von Säuren werden sie wieder ausgeschieden. Die beiden Säuren geben eine intensive Diazoreaktion; sie geben auch eine tief indigo-blaue Färbung durch Phosphomolybdänsäure und Ammoniak. Sie geben auch starke MILLOX'sche Reaktion. Kurzum, die charakteristischen Reaktionen des Roh-Oryzanins (I) werden durch diese beiden Säuren hervorgerufen.

Unter den Spaltungsprodukten des Roh-Oryzanins (I) fanden wir ausser diesen beiden Säuren noch ziemlich viel Cholin, und Traubenzucker, nebst einer organischen Säure, welche wir später als Nikotinsäure (m-Pyridincarbonsäure) identifiziert haben. Die Menge des Cholins und der Nikotinsäure scheint bei verschiedenen Kleiesorten sehr verschieden zu sein. Wenn die Mutterlauge der  $\alpha$ - und  $\beta$ -Säure mit Phosphowolframsäure versetzt wird, so entsteht ein weisser flockiger Niederschlag, der nach einiger Zeit auf den Boden sich absetzt. Nach Zerlegung dieses Niederschlages durch Baryt und Entfernung des überschüssigen Baryts mittels Schwefelsäure erhält man eine alkalisch reagierende Flüssigkeit, welche Cholin und Nikotinsäure enthält. Wird nun diese Flüssigkeit mit Pikrinsäure versetzt, so scheidet sich das Nikotinsäurepikrat zuerst aus und nach dem Einengen der Mutterlauge krystallisiert das Cholinpikrat als lange gelbe Prismen aus. Da das erstere in Wasser viel schwer löslich als das letztere ist, so lassen sich die beiden Pikrate leicht voneinander trennen. Die Ausbeute an Nikotinsäurepikrat beträgt ca. 0,5 g aus 4g Roh-Oryzanin (=1 kilo Kleie).

### I. Nikotinsäurepikrat.

Aus heissem Wasser scheidet sich das Pikrat als hellgelbe kurzen Stäbchen ab. Es schmilzt bei  $214^{\circ}$  (unkorr.) unter Zersetzung.

#### Analyse des Pikrates.

1.	0.1602 g Subst. gaben	0.2376 g $\text{CO}_2$	0.0348 g $\text{H}_2\text{O}$
	0.1291 g   "   "	19.0 c. c. N ( $21.5^{\circ}$ , 757 mm)	
	0.1076 g   "   "	0.0705 g Pikrinsäure.	

2)	0.1511 g	„	„	0.2254 g CO <sub>2</sub>	0.0350 g H <sub>2</sub> O		
	0.1342 g	„	„	18.2 cc. N (12°, 763.5 mm)			
				C	H	N	Pikrinsäure.
C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> , C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>	Ber.	40.91	2.27	15.91	65.06		
	Gef.	40.45	2.41	16.50	65.50		
		40.68	2.57	16.19	—		

Freie Nikotinsäure erhält man, indem das Pikrat im Wasser gelöst, mit wenig Schwefelsäure angesäuert, und wiederholt mit Aether geschüttelt wird, um die Pikrinsäure zu entfernen. Nach Entfernung der Schwefelsäure durch Baryt, dampft man die wässrige Lösung bei gelinder Wärme ein, bis die freie Nikotinsäure als farblosen Nadeln sich abscheidet. Nach einiger Zeit saugt man ab und wäscht mit wenig Alkohol und Aether.

Die freie Säure reagiert ziemlich stark sauer, wird durch Phosphorwolframsäure gefällt. Im Kapillarrohr erhitzt, schmilzt sie bei 228 bis 229° (unkorr.)

#### Analyse der freien Säure.

0,1394 g Subst. gaben	0,2983 g CO <sub>2</sub>	0,0542 g H <sub>2</sub> O	
0,0707 g „ „	7,05 c.c. N (14°, 764,5 mm)		
	C	H	N
C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> Ber.	58,54	4,07	11,38
Geb.	58,36	4,32	11,80

Ferner wurde das Kupfersalz sowie das Platinchloriddoppelsalz der Nikotinsäure dargestellt und analysiert. Alle diese Präparate waren mit denen aus reiner Nikotinsäure dargestellten vollständig identisch.

#### II. Cholinpikrat.

Cholinpikrat scheidet sich aus stark konzentrierter Mutterlauge des Nikotinsäurepikrates in grossen gelbbraunen Prismen aus. In Kapillarrohr erhitzt, verwandelt sich die gelbe Farbe bei ca. 100° ins Orangerote und zersetzt sich bei 240 (unkorr.) unter Schäumen. Die Ausbeute an Cholinpikrat beträgt im besten Falle ca. 2 g aus 4 g Roh-Oryzanin (I) (=1 kilo Kleie).



Die Analyse des im Vakuum bei 100° getrockneten Salzes gab folgende Zahlen:—

#### Analyse des Pikrates.

1.	0.1493 g Subst.	gaben	21,5 c.c. N (12° 756 mm)		
2.	0.4928 g	„ „	0.3414 g Pikrinsäure.		
			N	Pikrinsäure.	
	$C_5H_{14}NO$ .	$C_6H_2N_3O_7$	Ber.	16.87	68.97
			Gef.	17.00	69.28

Aus dem Pikrate wurde das Platinchloriddoppelsalz des Cholins dargestellt, indem das Pikrat in wenig Wasser suspendiert, mit wenig Salzsäure angesäuert und wiederholt mit Aether geschüttelt wurde, um die Pikrinsäure vollständig zu entfernen. Die farblose Lösung wurde nun stark eingeeengt und mit kleinem Ueberschuss von Platinchloridlösung versetzt. Es schied sich dabei das charakteristische Doppelsalz des Cholins aus, welches bei 230 bis 232° (unkorr.) unter Verkohlungs schmolz. Das Platindoppelsalz wurde einmal aus Wasser umkrystallisiert, im Vakuum bei 100° getrocknet und analysiert.

#### Analyse des Platinchloriddoppelsalzes des Cholins.

1.	0.2362 g Subst.	gaben	0.0736 g Pt.
			Pt.
	$(C_5H_{14}NO, Cl)_2$	$PtCl_4$	Ber.
			31.54
			Gef.
			31.54

Wir haben ferner aus reinem Cholin (Kahlbaum) das Pikrat und das Platinchloriddoppelsalz dargestellt und fanden, dass sie mit unserem Präparat vollständig identisch waren.

### III. Traubenzucker.

Aus dem Filtrat vom phosphowolframsauren Niederschlag der Niktinsäure und des Cholins haben wir nach dem Entfernen der Phosphowolframsäure durch Baryt eine reichliche Menge Glukose als Osazon



isoliert. Dieses Osazon war nach einmaligem Umlösen in heissem verdünntem Alkohol chemisch rein, hatte einen Schmelzpunkt von  $202^{\circ}$  (unkorr.) und zeigte die charakteristischen Krystallformen.

Wir haben auch die Menge der Spaltungsprodukte annähernd bestimmt. Aus 100 Theilen Roh-Oryzanin (I) wurden 10 T.  $\alpha$ - und  $\beta$ -Säure, 30 T. Cholin und Nikotinsäure, 23 T. Traubenzucker, ausserdem etwas harzartige schwarzbraune Substanz gewonnen.

1g Roh-Oryzanin gibt nach der Spaltung mit Säure

0.044 g Gesamtstickstoff,

0.035 g durch Phosphowolframsäure fällbaren Stickstoff,

0.009 g Stickstoff in anderer Form (hauptsächlich als  $\alpha$ - und  $\beta$ -Säure).

0.000 g Ammoniakstickstoff.

Da wir später unmittelbar aus Roh-Oryzanin (I) ebensoviel Nikotinsäurepikrat isolieren konnten, wie nach dem Erhitzen des ersteren mit verdünnter Schwefelsäure, so halten wir für wahrscheinlich, dass die letztgenannte Säure in der Kleie in freiem Zustande vorhanden ist.

Ob das Cholin auch als solches in der Kleie vorkommt, oder ob es erst nach der Erhitzen des Roh-Oryzanins mit Schwefelsäure entsteht, lässt sich nicht so leicht entscheiden. Es gelang uns jedenfalls nicht, unmittelbar aus Roh-Oryzanin (I) eine nennenswerte Menge des Cholinpikrates zu isolieren.

#### Weitere Reinigung des Oryzanins.

Zur weiteren Reinigung werden 4 g Roh-Oryzanin (I) in 100 c.c. Wasser gelöst und mit einer 20% igen wässerigen Tanninlösung so lange versetzt, bis nur noch schwache Trübung entsteht. Man braucht dazu 15 bis 20 c.c. Tanninlösung. Ein Ueberschuss von Tannin ist zu vermeiden. Die weisslichbraune, flockige Fällung wird abgesaugt, mit wenig 1% iger Tanninlösung rasch gewaschen. (Ein Ueberschuss ist zu vermeiden.) Der Niederschlag wird nun auf Tonplatte gestrichen, getrocknet und dann in einen Mörser gebracht, mit wenig Wasser verrieben. Man giebt nun so viel Aceton zu, bis der Niederschlag gelöst wird. Hierauf wird so viel

gesättigte Barytlösung zugegeben, bis die Flüssigkeit stark alkalisch reagiert, sorgfältig verrieben und abgesaugt. Der Rückstand wird noch zweimal mit Barytwasser verrieben und abgesaugt. Das verreinigte Filtrat wird nun mittels Schwefelsäure von überschüssigem Baryt befreit und im Vakuum eingedampft. Es bleibt dabei ein hellbrauner Syrup in kleiner Menge zurück, der nicht mehr sauer, sondern neutral oder manchmal schwach alkalisch reagiert. Wir bezeichnen diesen Syrup mit "Roh-Oryzanin (II)." Die Ausbeute ist sehr gering. Aus 4 g Roh-Oryzanin (I) wird durchschnittlich nur 0,25 bis 0,3 g erhalten. Dieses Präparat war nun dreimal so wirksam als Roh-Oryzanin (I). 0,01 g genügte schon, um eine erkrankte Taube zu heilen oder von Erkrankung zu bewahren.

Später haben wir dieses Verfahren etwas modifiziert und zwar in folgender Weise:—

Der Tanninniederschlag wird in einem Mörser mit 3% iger Schwefelsäure sorgfältig verrieben, abgesaugt und der Rückstand noch mehrere Male mit Schwefelsäure verrieben. Das Oryzanin geht dabei in Lösung über. Die gesamte Flüssigkeit wird nun mit einem Ueberschuss von Baryt versetzt, um Tannin und Schwefelsäure zu entfernen. Der dabei entstandene Niederschlag wird abgesaugt und das Filtrat davon wird, nach dem Entfernen des Baryts durch Schwefelsäure, bei verminderten Druck stark eingedampft, mit Aether geschüttelt und weiter eingeengt. In der Weise erhält man einen hellbraunen Syrup, der gewöhnlich wirksamer als Roh-Oryzanin (II) ist.

Wir haben auch öfters aus dem alkoholischen Extrakt der Kleie unmittelbar durch Tannin das Roh-Oryzanin gefällt und aus diesem Niederschlag durch weitere Behandlung mit 3% iger Schwefelsäure ein ziemlich wirksames Präparat dargestellt. Oder man kann umgekehrt das Präparat, das man unmittelbar durch Tannin-Verfahren erhalten hat, mit Phosphowolframsäure fällen.

Obgleich man in oben erwähneter Weise schon ein ziemlich wirksames Präparat erhalten konnte, konnte man es noch nicht als chemisch rein betrachten, so lange es noch nicht krystallisieren wollte.

Nach langen Bemühungen ist es uns geglückt, dies als Pikrinsäuresalz krystallinisch abzuscheiden. Wird eine konzentrierte wässrige Lösung des Roh-Oryzanins (II) mit wenig Pikrinsäure verrieben, so scheidet sich das Oryzaninpikrat als gelbbraune, flockige Fällung aus, die beim Abkühlen krystallinisch wird. Man saugt nun ab, wäscht mit wenig kaltem Wasser und trocknet über Schwefelsäure. Es bildet ein gelbbraunes Pulver. Da das Pikrat nicht leicht krystallisieren will, muss man nur sorgfältig arbeiten. Gibt man zu viel Pikrinsäure zu, so verwandelt sich das Pikrat zu einer braunen, weichen Masse, oder, erwärmt man die Lösung, so löst sich das Pikrat klar auf; nach dem Erkalten krystallisiert es jedoch nichtmehr aus. Man muss auch damit rechnen, dass das Roh-Oryzanin (II) immer noch etwas Nikotinsäure als Verunreinigung enthält; sie bildet auch ein Pikrat, welches sich schwer vom Oryzaninpikrat trennen lässt. Zu diesem Zwecke gibt man zuerst nur ungenügende Menge Pikrinsäure zu und verreibt in der Kälte (nicht erwärmen!); es scheidet sich das Oryzaninpikrat aus, während die Nikotinsäure in der Lösung zurückbleibt. Erst nach dem Erwärmen mit viel Pikrinsäure scheidet sich das Nikotinsäurepikrat aus. Zu weiterer Reinigung des Oryzaninpikrats, wird es in wenig kaltem Azeton gelöst, klar abfiltriert und über Schwefelsäure langsam eingedunstet. In der Weise erhält man das Pikrat als gelbbraune, mikroskopisch kleine Nadeln, welche sternförmig sich zusammengruppieren. (Taf. XIX. I.) Es löst sich in Aether und Petroläther nicht; in kaltem Wasser ist es ziemlich schwer, in heissem Wasser, Alkohol und Aceton aber leichter löslich.

Die Ausbeute an Pikrat war leider sehr schlecht, so sind wir noch nicht imstande, die genaue Beschreibung desselben und des freien Oryzanins zu geben. Ob es durch Spaltung  $\alpha$ - und  $\beta$ -Säure sowie Cholin, Traubenzucker etc. gibt, muss später untersucht werden.

### III. Tierversuche.

Bei der Wirkung des Oryzanins auf Tiere ist vor allem zu bemerken, dass z. B. die Tauben bei ausschliesslicher Reisfütterung binnen zwei bis drei Wochen etwa  $\frac{1}{3}$  des ursprünglichen Körpergewichtes

einbüßen und schliesslich zugrunde gehen. Eine gesunde Taube von 250 bis 300 g Körpergewicht frisst am Anfang 20 bis 30 g Reis täglich. Ungeschälter Reis oder geschälter Reis mit 3 g Kleie kann das Tier längere Zeit vor Erkrankung hüten, oder ein erkranktes in kurzer Zeit heilen.

Der alkoholische Extrakt, den wir in obenerwähnter Weise dargestellt haben, hat auch dieselbe Wirkung wie Kleie selbst, wenn täglich 0.3g (aus 3 g Kleie) per os oder mit Reis vermischt gegeben wird. Das Roh-Oryzanin (I), das wir durch das Phosphowölframsäureverfahren aus alkoholischem Extrakt dargestellt haben, war viel wirksamer als der alkoholische Extrakt selbst. 0,03g genügt um eine Taube vor Erkrankung zu hüten oder eine erkrankten zu heilen, d. h. 100 mal wirksamer als Kleie.

Das durch das Tanninverfahren weiter gereinigte Präparat ist wieder dreimal wirksamer als Roh-Oryzanin (I) und das reine Oryzanin, das wir als Pikrat krystallinisch erhalten haben, ist abermals doppelt so wirksam wie das letztere. 0,005 g sind ebenso wirksam wie 3 g Kleie oder 0,3 g alkoholischer Extrakt.

## A. Tauben.

### Versuch I.

#### Alkoholischer Kleieextrakt.

Hier wurde die Wirkung des alkoholischen Extrakts der Kleie auf Tauben geprüft. Zwei ausgewachsene Tauben wurden erst 4 Tage mit geschältem Reis gefüttert, die nächsten 16 Tage, also von 5 tens bis 21 ~~tens~~ bekamen sie täglich 0,3 g alkoholischen Extrakt dazu; und weitere 15 Tage wieder Reis allein. So lange sie mit alkoholischem Extrakt versehen wurden, blieben sie gesund und munter und nahmen etwas an Gewicht zu; aber bald nachdem der alkoholische Extrakt eingestellt wurde, fingen sie an abzumagern, verloren nach und nach an Gewicht und erkrankten endlich. Hierauf bekamen sie wieder täglich 0,3 g alkoholischen Extrakt. Schon am nächsten Tage erholten sie sich erheblich. Die

Esslust kam wieder zurück und sie erreichten das ursprüngliche Gewicht. Gegen Ende des Versuches haben die Versuchstiere 18 Tage lang bloss destilliertes Wasser anstatt gewöhnlichen Brunnenwassers bekommen, ohne irgend eine Störung zu zeigen.

Aus diesem Versuchen kann man schliessen, dass der alkoholische Extrakt für die Erhaltung des tierischen Lebens absolut notwendig ist, falls die Tiere ausschliesslich mit Reis gefüttert wurden.

### Versuch II.

Geschälter Reis mit Roh-Oryzanin (I) und Salzen.

Mit 1000 g geschältem Reis wurden 1,2 g Roh-Oryzanin (I), 3,4 g Lecithin (Kahlbaum), 4,3 g Phytin, 2,6 g  $\text{CaCO}_3$ , 0,85 g  $\text{CaCl}_2$  und 1,7 g  $\text{Na}_2\text{CO}_3$  vermischt und an zwei Tauben verfüttert. Sie waren 17 Tage vollkommen gesund und nahmen an Körpergewicht zu. Nach 17 Tagen wurde der Versuch unterbrochen.

Tabelle I.

Versuchstage	Körpergewicht	
	(1)	(2)
1	312	249
3	329	259
5	338	264
7	337	271
9	333	271
11	336	268
13	338	272
15	336	272
17	336	273

## Versuch III. (Taf. XX.)

## Roh-Oryzanin (I).

Zwei Tauben wurden zuerst mit geschältem Reis, Lecithin, Phytin- und Salzgemischen gefüttert. Nach 14 Tagen waren sie ermattet und erkrankt. Nun wurde täglich 0,03 g Roh-Oryzanin (I) per os gegeben. Schon am nächsten Tage waren sie beinahe geheilt; der Appetit kam wieder zurück. Nach 3 Tagen waren sie vollkommen gesund; das Körpergewicht nahm auch allmählich zu. Nach 17 Tagen wurde der Versuch unterbrochen.

Tabelle II.

Versuchstage	Körpergewicht		Bemerkungen
	(1)	(2)	
1	272	305	Ohne Oryzanin Erkrankt
14	229	256	
15	214	222	Geheilt  Mit 0.03 g Roh-Oryzanin (I) täglich  Vollständig gesund
16	229	232	
17	242	233	
19	241	248	
21	242	266	
23	231	267	
25	237	267	
27	242	278	
29	247	281	
31	245	286	

## Versuch IV. (Taf. XX.)

Dass das Oryzanin zum grössten Teil durch Phosphowolframsäure aus dem alkoholischen Extrakt mitgerissen wird, wird dadurch bewiesen, dass das Filtrat vom Phosphowolframsäureniederschlag nur noch schwache

Wirkung hat. Wenn man das Filtrat vom Phosphowolframsäureniederschlag durch Zusatz von Barythydrat von überschüssiger Phosphowolframsäure und Schwefelsäure befreit, und den Überschuss vom Baryt wieder durch verdünnte Schwefelsäure vollständig entfernt, klar abfiltriert und unter vermindertem Druck verdampft, so erhält man einen dickflüssigen braunen Syrup, der auf erkrankte Tauben keine Wirkung hat. Die Taube war nicht damit geheilt; sondern wurde immer schwächer. Nach 6 Tagen wurden 0,02 g Roh-Oryzanin per os gegeben. Die Wirkung war überraschend. Nach zwei Tagen war die Taube schon gesund und nach 9 Tagen hatte sie an Körpergewicht um 38 g zugenommen. Nach Einstellen der Oryzaninzugabe magerte das Tier wieder allmählich ab und erkrankte.

Tabelle III.

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	1	289	Gesund
	4	277	
	6	280	
	7	278	
	8	261	
	9	253	
	10	250	
	11	238	
	12	232	Erkrankt
	13	233	
Filtrat vom Phosphowolframsäureniederschlag	14	226	*0.03 g Roh-Oryzanin (1) (nur einmal gegeben)
	15	227*	
	16	243	
	17	236	
	18	226	
	19	230	Erkrankt



	Versuchstage	Körpergewicht	Bemerkungen
Filtrat vom Phosphorwolframsäure-niederschlag	20	224	Geheilt
	21	235	
	22	236	
	23	247	0.03 g Roh-Oryzanin (I) täglich
	24	260	
	25	255	
	26	256	
	27	260	
	28	262	Gesund
	29	261	Noch gesund
	30	261	
	31	261	
	32	263	
	33	254	
	34	250	
	35	245	
	36	242	Allmählich schwach

## Versuch V. (Taf. XX.)

## Roh-Oryzanin (II).

Zu zwei Tauben, die vorher bei der Reisfütterung erkrankt waren, wurde 0,01 g Roh-Oryzanin (II), am ersten Tagen subcutan eingespritzt und vom zweiten Tagen an per os gegeben. Sie waren ebenso schnell geheilt wie mit dem alkoholischen Extrakt. Nach 6 bzw. 7 Tagen bekamen sie wieder den Reis allein, trotzdem blieben sie noch mehrere Tage gesund und munter.



Tabelle IV.

( 1 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	:	296	Gesund
	:	299	
	:	220	Erkrankt
	:	223	
0.01g Roh-Oryzanin (II)	1	220	Geheilt
	2	235	
	3	241	
	4	235	Gesund
	5	239	
	6	242	
Reis allein	7	254	Allmählich schwach
	8	250	
	9	247	
	10	245	
	11	242	

( 2 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	:	270	Gesund
	:	278	
	:	292	
	:	292	
	:	290	
	:	227	Erkrankt
	:	223	

	Versuchstage	Körpergewicht	Bemerkungen
0.01g Roh-Oryzanin (II)	1	217	Geheilt
	2	227	
	3	222	
	4	234	Gesund
	5	239	
	6	242	
	7	244	
Reis allein	8	244	Noch nicht erkrankt
	9	245	
	10	245	
	11	245	
	12	240	
	:		

Die Fällung des Oryzanins durch Tannin ist nur eine unvollständige. Bloss aus konzentrierter Lösung ruft Tannin Fällung hervor, die sowohl durch Verdünnung mit Wasser, als auch Zusatz von verdünnten Säuren und sogar durch einen Überschuss von Tannin wieder gelöst wird. Ferner, während der Verarbeitung des Tanninniederschlags geht ein Teil Oryzanin verloren, so dass die Ausbeute nur eine sehr geringfügige ist. Einmal haben wir versucht, aus dem Filtrate des Tanninniederschlags Oryzanin zu gewinnen. Zu diesem Zwecke wurde 1 g Roh-Oryzanin (I) in 100 c.c. Wasser gelöst und mit einer 20% igen Tanninlösung gefällt. Das Filtrat von Tanninniederschlag wurde mit Barythydrat versetzt, bis die Flüssigkeit stark alkalisch reagierte. Die dicke weissliche Fällung, die eine grünlich braune Farbe annahm, wurde abgesaugt und mit wenig Wasser gewaschen. Das klare Filtrat wurde nun mit verdünnter Schwefelsäure sorgfältig vom Baryt befreit, abfiltriert und unter vermindertem Druck abdestilliert. Der dabei zurückgebliebene hellbraune Syrup, der ziemlich sauer reagierte wurde im Exsiccator über Schwefelsäure getrocknet. In diesem Präparate war kein Oryzanin mehr vorhanden. Einmal

haben wir 0,04 g davon einer erkrankten Taube gegeben. Das Tier war nicht damit geheilt und das Körpergewicht ging allmählich herunter. Erst durch Verabreichung von 0,01 g Roh-Oryzanin (II) wurde es wieder geheilt. Es scheint also, dass der Hauptanteil des Oryzanins in Roh-Oryzanin (I) durch Tannin gefällt worden und was noch im Filtrat geblieben war, durch weitere Verarbeitung verloren gegangen ist.

Zweite Versuchsreihe mit Roh-Oryzanin (II).

Hier wurde das Oryzanin durch Tannin fraktioniert gefällt. 1g Roh-Oryzanin (I) wurde in 100c.c. Wasser gelöst und zuerst mit 10c.c. einer 20% igen Tanninlösung versetzt. Der Niederschlag (a) wurde abgesaugt. Das Filtrat wurde wieder mit 10 c.c. Tanninlösung versetzt. Es entstand noch eine Fällung (b). Das Filtrat von (b) gab keine Fällung mehr.

Die beiden Niederschläge (a) und (b) wurden in oben angegebener Weise mit Baryt zerlegt und daraus das freie Oryzanin dargestellt. Das Präparat aus dem Niederschlag (a) war viel wirksamer als dasjenige aus (b). 0,01 g des ersteren konnte eine erkrankte Taube in wenigen Tagen heilen; während das letztere nur langsam seine Wirkung entfaltete.

Tabelle V.

( 1 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein		283	Gesund
		⋮	
		⋮	
0,01g Roh-Oryzanin(II)(a)	1	210	Erkrankt
	2	212	
	3	226	Geheilt
	4	218	
	5	217	
	6	220	Gesund

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	7	220	Gesund
	8	219	
	9	226	
	10	227	
	11	227	
	12	223	
	13	229	Noch nicht erkrankt
	14	224	
	15	225	
	16	223	
	17	223	
	18	226	
	19	220	Allmählich schwach
	20	217	
	21	208	

( 2 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	:	270	Gesund
	:	268	
	:	240	
	:	240	
	:	236	
	:	234	
	:	227	
	:	225	
	:	223	Erkrankt
	:	219	
	:		

	Versuchstage	Körpergewicht	Bemerkungen
0,01 g Roh-Oryzanin (II) (b)	1	215	
	2	217	
	3	219	
	4	223	Geheilt
	5	224	Gesund
	6	225	
	7	224	
	8	226	
	9	228	
	10	226	
	11	228	
	12	225	
	13	230	
	14	232	
	15	235	
	16	241	
	17	239	Körpergewicht allmählich steigend

Ein ziemlich wirksames Präparat kann man auch unmittelbar aus alkoholischem Extrakt durch Füllen mit Tannin erhalten. Zu diesem Zwecke löst man den alkoholischen Extrakt in möglichst wenig Wasser und gibt so viel Tanninlösung zu, bis nur noch schwache Trübung entsteht. Der braune Niederschlag wird in gewöhnlicher Weise mit Baryt zerlegt und weiter verarbeitet. Die Wirkung des so bereiteten Präparates war nicht immer konstant. 0,01 g desselben reichte jedoch in vielen Fällen aus, um eine erkrankte Taube zu heilen obgleich es nur langsam wirkte.

Tabelle VI.

( 1 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	·	286	Gesund
	·	·	·
	·	246	Erkrankt
0,01 g Roh-Oryzanin	1	241	Geheilt
	3	249	
	5	244	
	7	246	
	9	256	
	11	254	
	13	255	
	15	254	Gesund
	17	253	
	19	256	

( 2 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	·	253	Gesund
	·	·	·
	·	201	Erkrankt
0,01 g Roh-Oryzanin	1	201	Nur langsam geheilt
	3	203	
	5	202	
	7	202	
	8	203	
0,02 g Roh-Oryzanin	9	209	Gesund
	11	213	
	13	210	Körpergewicht steigt
	15	215	

## Versuch VI. (Taft. XXI.)

Der Tanninniederschlag selbst war auch wirksam. Da der Niederschlag nicht in Wasser löslich war, wurde er in verdünnter Natronlauge gelöst und gegeben. 0,03 g desselben genügte schon um eine erkrankte Taube zu heilen.

Tabelle VII.

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	:	:	Gesund
	:	:	
	:	:	Erkrankt
0,03 g Tanninniederschlag	1	189	
	3	190	
	5	196	Geheilt
	7	196	
	9	200	
	11	202	
	13	204	
	15	208	Gesund
Reis allein	17	209	
	19	213	
	21	216	
	23	211	Noch gesund
	25	209	
	27	207	
	29	204	Allmählich schwächer

## Versuch VII. (Taf. XXI.)

## Reines Oryzanin.

Das reine Oryzanin, das wir aus dem Pikrate in oben erwähnter Weise dargestellt haben, wurde nur in ganz geringer Menge gewonnen und reichte für längere Versuche nicht aus, deshalb haben wir es einmal einer erkrankten Taube nur 4 Tage lang und einer zweiten nur 3 Tage lang gegeben und zwar der ersten 0.01g und der zweiten 0.005g täglich. Die Wirkung war trotzdem sehr deutlich; sie waren rasch geheilt und blieben mehr als 10 Tage gesund und munter. Das Körpergewicht stieg auch sehr hoch. Erst nach 10 Tage ging es wieder langsam zurück.

Tabelle VIII.

( 1 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	:	:	Gesund
	:	:	
	:	240	
	:	243	
	:	239	
	:	232	
	:	232	
	:	224	
	:	222	Allmählich appetitlos
Filtrat v. Pikraten aus Roh- Oryzanin (II)	1	216	Fast keine Wirkung  Erkrankt
	2	221	
	3	217	
	4	212	
	5	215	
	6	210	
	7	212	
		209	



	Versuchstage	Körpergewicht	Bemerkungen
	9	213	Schwach
0,02 g Pikrat=ca. 0.01 g Orysanin	10	207	
	11	217	Geheilt und munter
	12	218	
	13	225	Gesund
Reis allein	14	233	
	15	245	
	16	238	
	17	243	
	18	245	Gesund
	19	253	
	20	260	
	21	247	
	22	253	
	23	252	
	24	242	Noch gesund

( 2 )

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	:	:	Gesund
	:	235	
	:	225	
	:	226	Erkrankt
0,01 g Pikrat=0,005 g Oryzanin	1	228	
	2	215	
	3	225	Geheilt

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	4	225	Gesund
	5	227	
	6	235	
	7	228	
	8	243	
	9	240	Gesund
	10	240	
	11	234	
	12	232	Allmählich Appetit verloren
	13	225	

## Versuch VIII. (Taf. XXII.)

## Gemischtes Futter.

Vier Tauben wurden anstatt mit Reis, mit einem künstlich-gemischtem Futter gefüttert. Die Mengenverhältnisse waren wie folgt:—

Stärke ... ..	500,0 g	CaCO <sub>3</sub> ... ..	1,5 g
Pepton ... ..	25,0 g	CaCl <sub>2</sub> ... ..	0,5 g
Lecithin... ..	2,5 g	K <sub>2</sub> CO <sub>3</sub> ... ..	0,5 g
Phytin ... ..	2,5 g	Na <sub>2</sub> CO <sub>3</sub> ... ..	1,0 g

Die Stärke wurde vorher verkleistert, mit den übrigen Stoffen vermischt, bei niedriger Temperatur getrocknet und in kleine Stückchen geschnitten. Die ersten zwei (1) und (2) bekamen kein Oryzanin, während den anderen zwei (3) und (4) täglich 0.03 g Roh-Oryzanin per os gegeben wurden. Der Unterschied zwischen beiden war auffallend; (1) und (2) die kein Oryzanin bekamen, gingen in 10 bzw. 11 Tagen unter starker Abmagerung zugrunde; während (3) und (4), die Oryzanin bekommen haben, nicht nur gesund blieben, sondern in 17 Tagen an Körpergewicht um 74 bzw. 42 g zunahmen.

Tabelle IX.

(A) Ohne Oryzanin

	Versuchs- tage	Körpergewicht u. Nr. d. Versuchstieres		Bemerkung
		(1)	(2)	
Reis allein		·	·	
Stärke, Pepton, Lecithin Salze	1	219	207	Gesund
	2	213	209	
	3	226	207	
		208	196	
	5	208	192	
	6	200	191	Erkrankt
	7	201	190	
	8	188	180	
	9	180	176	
	10	195	190	
	11	starb	170	
	12		starb	

(B) Mit Oryzanin

	Versuchs- tage	Körpergewicht u. Nr. d. Versuchstieres		Bemerkungen
		(1)	(2)	
Reis allein				
Stärke, Pepton, Lecithin, Salze mit Oryzanin	1	250	243	Gesund
	2	269	262	
	3	280	243	
	4	279	255	
	5	274	255	

	Versuchstage	Körpergewicht u. Nr. d. Versuchstieres		Bemerkungen
		(1)	(2)	
Stärke, Pepton, Lecithin, Salze mit Oryzanin	6	287	264	
	7	285	282	
	8	285	289	
	9	293	288	
	10	279	290	
	11	286	291	
	12	284	299	
	13	290	300	
	14	290	303	
	15	296	307	
	16	300	317	Gesund
	17	292	317	Körpergewicht steigt

## Versuch IX. (Taf. XXII.)

## Gemischtes Futter.

In diesem Versuche wurde das Pepton durch Gelatine ersetzt. Das Futtergemisch hatte folgende Zusammensetzung:—

## A. Ohne Oryzanin.

Stärke	...	...	...	500,0 g
Gelatine	...	...	...	52,0 g
Lecithin	...	...	...	2,5 g
Phytin	...	...	...	2,5 g
CaCO <sub>3</sub>	...	...	...	1,5 g
CaCl <sub>2</sub>	...	...	...	0,5 g
Na <sub>2</sub> CO <sub>3</sub>	...	...	...	1,0 g
K <sub>2</sub> CO <sub>3</sub>	...	...	...	0,5 g

## B. Mit Oryzanin.

A+10 g alkoholischer Extrakt der  
Kleie.

Hier beobachtete man auch einen auffallend grossen Unterschied zwischen (A) und (B). Zwei Tauben, die mit (A) gefüttert wurden, hatten von Anfang an keine Esslust. Das Körpergewicht hat so rasch abgenommen, dass sie nach 12 Tagen schon stark abgemagert und

erkrankt waren. Die zwei anderen Tauben, die mit (B) gefüttert wurden, konnten auch nicht das Gleichgewicht behalten und das Körpergewicht nahm auch allmählich ab. Trotzdem waren sie nach 28 Tagen noch gesund. Der durchschnittliche Verlust an Körpergewicht betrug bei (A) 6,9 bezw. 5,2 g und bei (B) 1,4 bezw. 2,0 g. Also bei (A) war der Verlust 3 bis 4 mal grösser als bei (B). Dieser Unterschied ist schlechthin dem Oryzanin zuzuschreiben.

Die allgemeine Annahme, dass das Gelatin nicht das Eiweiss, wie Kasein, Pepton etc. ersetzen kann, ist wiederum hier bestätigt.

Tabelle X.  
(A) Ohne Oryzanin

	Versuchstage	Körpergewicht u. Nummer des Tieres		Bemerkungen
		(1)	(2)	
Stärke, Gelatin, etc.	1	296	310	Gesund
	3	285	287	
	5	270	270	
	7	257	253	
	9	249	244	Erkrankt
	11	236	228	
	13	226	221	
	15	218	207	

Täglicher Verlust an Gewicht bei A:

(1) 6,9 g, (2) 5,2 g.

(B) Mit Oryzanin

	Versuchstage	Körpergewicht u. Nummer des Tieres		Bemerkungen
		(1)	(2)	
Stärke, Gelatine, etc. mit Oryzanin	1	253	262	Gesund
	3	231	214	
	5	217	220	

	Versuchs- tage	Körpergewicht u. Nummer des Tieres		Bemerkungen
		(1)	(2)	
Stärke, Gelatine usw. mit Oryzanin	7	239	217	
	9	235	216	
	11	232	214	
	13	235	212	
	15	228	210	
	17	225	204	
	19	226	203	
	21	221	206	
	23	219	202	
	25	220	202	
	27	213	205	Körpergewicht geht allmählich herunter
	28	215	205	

Täglicher Verlust bei B:

(1) 1,4 g, (2) 2,0 g

### Versuch X. (Taf. XXII.)

#### Spaltungsprodukte des Eiweisses.

Wie oben erwähnt, verlieren die Tauben ihr Körpergewicht nur langsam, wenn sie mit eiweissfreiem aber oryzaninhaltigem Futter genährt werden. Darum haben wir versucht, ob das Aminosäurengemisch, das durch Spaltung des Eiweisses rein dargestellt wurde, den Verlust an Körpergewicht zu verhindern vermag.

Das Futtermisch hatte folgende Zusammensetzung:

Stärke ... ..	1000 g	$\text{Na}_2\text{CO}_3$ ... ..	2 g
Lecithin ... ..	5 g	$\text{CaCl}_2$ ... ..	1 g
Phytin ... ..	5 g	Alkoholische Extrakt d. Kleie	20 g
$\text{CaCO}_3$ ... ..	3 g		

Die Stärke wurden vorher verhleistert, mit übrigen Stoffen vermisch, getrocknet, zu kleinen Stückchen geschnitten und zwei Tauben gegeben.

Das Aminosäuregemisch bestand aus reinem Glykokoll, Alanin, Leucin, Phenylalanin, Histidinhydrochlorat, Asparaginsäure, Tyrosin, Cystin und Glutaminsäurehydrochlorat. Sie wurden ungefähr in derselben Mengenverhältnisse gemischt, wie sie in gewöhnlichen Eiweissmolekulan vorhanden sind. Leider haben wir kein reines Tryptophan und Prolin zur Hand gehabt, und so ist dieser Versuch nicht als endgültig anzusehen. Wir beabsichtigen später nochmals diesen Versuch zu wiederholen.

Zwei Tauben wurden erst 33 Tage lang mit dem oben angegebenen Futtergemisch genährt. Sie haben dabei allmählich an Körpergewicht verloren und waren beinahe erkrankt. Hierauf wurde vom 34. bis 49. Tage täglich 0,3 g Aminosäuregemisch verabreicht; sie konnten das Gleichgewicht nicht behalten, obgleich die Abnahme des Gewichts viel langsamer geworden ist. Vom 50. bis 80. Tage war die Aminosäuremenge auf 5.0 g vermehrt, die Abnahme wurde dabei noch langsamer und 14 Tage lang haben sie sogar beinahe das Gleichgewicht behalten. Später nahm es allmählich ab und nach 95 Tagen ging das Tier schliesslich zugrunde.

Ogleich die Aminosäuren in diesem Versuch nicht imstande waren, das Eiweiss zu ersetzen oder längere Zeit das Gleichgewicht zu behalten, haben die Tiere trotzdem viel länger als ohne Aminosäuren gelebt, und so muss man annehmen, dass sie wenigstens den Eiweissverbrauch im Tierkörper vermindert oder einen Teil des Eiweisses ersetzt haben.

Tabelle XI.

	Versuchstage	Körpergewicht u. Nr. des Tieres		Bemerkungen
		(1)	(2)	
Stärke, Lecithin, Phytin, Salze ohne Aminosäuren	1	254	276	Gesund
	3	251	277	
	5	248	272	
	7	247	265	
	9	241	259	
	11	232	256	
	13	231	251	
	15	229	250	
	17	228	246	
	19	220	245	
	21	217	240	
	23	214	239	
	25	211	—	
	27	213	230	
	29	207	224	
0.3 g Aminosäuren dazu	31	205	224	Allmählich schwach
	33	200	211	
	35	201	210	
	37	197	214	
	39	199	213	
	41	199	205	
	43	194	203	
	45	197	201	
	47	199	203	
	49	204	201	



	Versuchst- tage	Körpergewicht u. Nr. des Tieres		Bemerkungen
		(1)	(2)	
0.5 g Aminosäuren dazu	51	217	200	(1) Starb wegen mecha- nischer Verstopfung durch Anhäufung der Exkre- mente. Die angebliche Zunahme des Gewichts n. 50 Tagen ist wohl d. An- häufung des Exkrements zuzuschreiben
	53	211	199	
	55	218	202	
	57	223	195	
	59	243	199	
	61	starb	198	
	63		200	
	65		193	
	67		192	
	69		190	Allmählich schwach
	71		187	
	73		180	
	75		186	
	77		181	
	79		180	
	81		187	
	83		182	
	85		175	
	87		174	
	89		174	
	95		starb	

## B. Hühner.

## Versuch I.

## Geschälter Reis.

(1) Zwei junge Hühner (2 Monate nach dem Ausbrüten) wurden mit geschältem Reis gefüttert. Nach 10 bis 13 Tagen waren die beiden schon stark abgemagert. Am 15 ten haben sie je 1 g Pepton mit kleiner

Menge Phytin, Ferratin und Salzgemisch ( $\text{CaCO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{NaCl}$ ) bekommen. Sie waren aber nicht damit geheilt und gingen bald zugrunde.

Tabelle XII.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	481	537
17	206 starb	—
20	—	323 starb

(2) Derselbe Versuch wurde nochmals wiederholt.

Tabelle XIII.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	428	315
15	307 starb	—
18	—	260 starb

## Versuch II.

## Geschälter Reis mit Phytin.

1. Zwei junge Hühner (70 Tage alt) wurden mit geschältem Reis gefüttert; man gab täglich 0,5 g Phytin dazu. Sie waren nach 18 Tagen stark abgemagert und erkrankten schliesslich,

Tabelle XIV.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	482	418
18	356	307

2. Zwei andere Hühner (70 Tage alt) bekamen eine kleine Menge Salz nebst Phytin. Sie waren ebenso schnell erkrankt wie mit Reis allein.

## Versuch III.

## Geschälter Reis mit Lecithin.

Zwei jungen Hühnern wurde täglich 0,5g Lecithin (Kahlbaum) verabreicht. Die Abnahme des Körpergewichts war ebenso schnell wie mit Reis allein. Am 13 ten Tage waren sie schon schwer erkrankt. Hierauf wurde 5 g Kleie gegeben. Am nächsten Tage waren sie beinahe geheilt und bekamen wieder Esslust. Nach 12 Tagen waren sie vollständig gesund und hatten an Gewicht zugenommen.

Tabelle XV.

	Versuchstage	Körpergewicht u. Nr. des Tieres		Bemerkungen
		(1)	(2)	
Reis+Lecithin	1	413	497	Gesund
	3	390	492	
	5	372	462	
	7	355	443	
	9	341	408	
	11	336	388	
	13	306	375	Erkrankt

	Versuchstage	Körpergewicht u. Nr. des Tieres		Bemerkungen
		(1)	(2)	
5 g Kleie dazu	15	285	390	Geheilt
	17	325	404	
	19	319	412	
	21	363	406	
	23	331	445	
	24	365	431	Gesund

## Versuch IV.

Geschälter Reis mit alkoholischem Extrakt der Kleie und Salzen:  
Zusammensetzung des Futtermittels:

Geschälter Reis	1000 g	$\text{Na}_2\text{CO}_3$	2 g
Alkoholischer Extrakt	20 g	$\text{K}_2\text{CO}_3$	1 g
$\text{Ca}_3\text{H}_2(\text{PO}_4)_2$	5 g	$\text{NaCl}$	2 g
$\text{CaCO}_3$	3 g		

2 junge Hühner (ca. 25 Tage nach dem Ausbrüten) wurden mit obenerwähntem Futtermittel gefüttert. Anfangsgewicht war 156 bzw. 144 g. Sie blieben 70 Tage lang gesund und haben allmählich an Gewicht zugenommen, bis sie ungefähr das Doppelte ihres Gewichts erreicht hatten. Nach 80 Tagen waren sie jedoch schwach geworden, so dass wir den Versuch unterbrechen mussten.

Wir haben noch einige Versuche ausgeführt, um zu sehen, ob die jungen Hühner ausschliesslich mit geschältem Reis und alkoholischem Extrakt der Kleie längere Zeit am Leben bleiben können. In allen bisher untersuchten Fällen blieben sie 60 bis 70 Tage lang gesund und munter. Nachher ging der Appetit allmählich zurück. Sie starben nicht so schnell wie mit Reis allein, sondern blieben längere Zeit kümmerlich. Das Körpergewicht nimmt nach und nach ab. Da dem Reis fast alle unentbehrlichen Mineralstoffe fehlen, so muss man genau die Mengen-

verhältnisse und Verbindungsformen der einzelnen Mineralstoffe kennen lernen, um die jungen Hühner längere Zeit am Leben zu erhalten.

Wir wollen hier einige Beobachtungen, die Herr Direktor Kozai<sup>1</sup> und Dr. Ando in Nishigahara in dieser Richtung gemacht haben, erwähnen.

Sie haben eine Anzahl ausgewachsener Hühner in einem beschränkten Raume mit verschiedenen Futtermitteln gefüttert und gefunden, dass ungeschälter Reis oder geschälter Reis mit Kleie oder Weizenkleie nie die Erkrankung oder Abmagerung des Tieres hervorrief. Alle Tiere, die mit geschältem Reis gefüttert wurden, gingen ohne Ausnahme zugrunde. Bloss die Zeit bis zur Erkrankung war bei ihnen bedeutend länger als bei unserem Experimente, weil sie anfangs den käuflichen geschälten Reis ohne Waschen den Tieren gegeben haben, wobei natürlich kleine Mengen Kleie immer noch darauf haften blieben. Mit gewaschenem Reis erkrankten die Tiere viel rascher.

Die beiden Autoren haben auch verschiedene Reissorten aus verschiedenen Gegenden verglichen; sie verhielten sich manchmal sehr verschieden gegen Erkrankung, vorausgesetzt, dass der geschälte Reis nicht gewaschen war. Wenn er aber sorgfältig gewaschen und von Spuren anhaftender Kleie befreit war, so haben sie ohne eine einzige Ausnahme Erkrankung hervorgerufen.

Die alten ausgewachsenen Hühner waren viel widerstandsfähiger als die jungen wachsenden.

Ferner muss man bemerken, dass die Hühner, wenn sie in einem etwas geräumigen Raume gefüttert werden, nicht selten Fliegen, Mücken u. a. herunterschlucken, oder sie finden etwas, was sie nicht fressen sollten, so werden sie mehr oder weniger länger vor der Erkrankung verschont.

1. Special Report of the Agricultural Experiment Station Nishigahara, Tokio.

## C. Mäuse.

## Versuch I. (Taf. XXIII.)

## Geschälter Reis.

a) 4 Mäuse wurden mit geschältem Reis und Brunnenwasser gefüttert. Die ersten 4 bis 5 Tage waren sie gesund und frassen den Reis sehr gerne. Der Appetit ging aber nach und nach zurück, und binnen 11 bis 15 Tagen gingen sie alle zugrunde.

Tabelle XVI.

Versuchstage	Körpergewicht und Nummer des Tieres			
	(1)	(2)	(3)	(4)
1	8,1	10,9	7,2	7,2
4	8,5	10,0	6,8	7,4
7	7,9	9,9	6,4	6,6
9	7,9	9,2	6,2	6,4
11	7,2	8,5	5,7	5,7
13	6,6	8,1	5,7	starb
15	starb	starb	starb	—

b) Derselbe Versuch wurde nochmals mit zwei grösseren Mäusen wiederholt. Die beiden Tiere starben binnen 15 bis 17 Tagen unter Abmagerung.

Tabelle XVII.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	20,5	13,1
3	20,7	13,8
5	20,5	14,2

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
7	19,6	13,8
9	19,4	13,3
11	18,2	12,8
13	17,8	12,1
15	16,9	11,4
17	15,0 starb	starb

## Versuch II. (Taf. XXIII.)

## Ungeschälter Reis.

4 Mäuse wurden mit ungeschältem Reis gefüttert; sie blieben alle gesund. Jedes Tier frass täglich 2 bis 2,5 g Reis und nahm mehr oder weniger an Körpergewicht zu. Nach 33 Tagen wurde der Versuch unterbrochen.

Tabelle XVIII.

Versuchstage	Körpergewicht und Nummer des Tieres			
	(1)	(2)	(3)	(4)
1	9,1	9,4	8,1	5,9
4	10,1	10,3	9,0	6,2
6	10,4	10,4	8,9	6,4
8	10,6	10,5	8,7	6,2
10	10,5	10,6	8,9	6,2
12	10,5	10,8	9,2	6,2
14	10,8	10,5	9,0	6,4
17	11,2	11,1	9,7	6,2

Versuchstage	Körpergewicht und Nummer des Tieres			
	(1)	(2)	(3)	(4)
19	11,3	10,8	9,4	6,2
22	11,1	10,8	9,3	6,5
24	10,4	11,0	8,7	6,6
27	10,9	10,8	8,9	6,8
29	11,0	11,1	8,1	7,0
31	11,5	11,6	8,5	7,0
33	10,8	11,4	8,2	6,9

## Versuch III. (Taf. XXIII.)

Geschälter Reis mit alkoholischem Extrakt der Kleie.

a). 500 g geschälter Reis wurden mit einer wässerigen Lösung von 7 g alkoholischen Extrakt (=aus 70 g Kleie) vermischt, getrocknet und 3 Mäusen gegeben. Sie blieben gesund und normal. Nach 44 Tagen wurde der Versuch unterbrochen. Das Körpergewicht zeigte keine grossen Schwankungen.

Tabelle XIX.

Versuchstage	Körpergewicht und Nummer des Tieres		
	(1)	(2)	(3)
1	9,6	7,5	8,4
3	9,8	7,6	8,6
5	10,2	7,9	9,0
7	10,2	8,2	8,9
10	10,0	8,2	8,6
12	10,9	7,7	8,6
14	10,6	7,5	8,8



Versuchstage	Körpergewicht und Nummer des Tieres		
	(1)	(2)	(3)
16	10,1	7,4	8,9
18	9,6	7,4	8,5
22	9,2	7,3	8,7
24	11,2 (?)	7,4	8,9
28	9,3	7,4	8,9
30	9,4	7,2	8,8
32	9,6	7,2	8,3
35	9,2	7,0	8,4
37	9,1	7,1	8,8
44	9,2	7,2	8,7

b) Derselbe Versuch wurde nochmals mit 2 jungen Mäusen wiederholt. 80 Tage lang waren sie vollkommen gesund und das Körpergewicht nahm bedeutend zu. Nach 80 Tagen wurden der Versuch unterbrochen.

Tabelle XX.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	8,7	9,4
5	9,6	11,1
9	11,5	11,7
13	11,8	11,3
17	9,8	11,2
21	10,3	11,2
25	10,3	11,1
29	11,6	11,3

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
33	12,1	11,6
37	13,2	11,6
41	13,6	12,0
45	13,8	12,1
49	13,8	12,0
53	13,8	12,1
57	13,8	11,9
61	13,7	12,0
65	13,5	12,0
69	14,5	12,7
71	14,5	12,3
73	14,3	11,9
75	14,5	12,7
76	14,0	12,3
78	13,5	12,0
80	13,6	11,5

c) In diesem Versuche wurden 2 Mäuse mit geschältem Reis und alkoholischem Extrakt nebst kleinen Mengen Salzen gefüttert. Die Mengenverhältnisse waren wie folgt:

100 g geschälter Reis,  
 1,5 g alkoholischer Extrakt,  
 0,1 g  $K_2CO_3$ ,  
 0,2 g  $Na_2CO_3$ ,  
 0,1 g  $CaCl_2$ ,  
 0,3 g  $CaCO_3$ .

Die Versuchstiere blieben längere Zeit vollständig gesund. Das eine ist leider nach 57 Tagen weggelaufen. Das andere blieb 75 Tage lang ganz normal, bis der Versuch unterbrochen wurde.

Tabelle XXI.

Versuchstage	Körpergewicht und Nummer des Thieres	
	(1)	(2)
1	9,0	10,0
5	10,7	11,3
9	13,0	12,9
13	13,0	12,8
17	12,7	12,6
21	13,6	12,9
25	13,0	13,2
29	12,9	13,8
33	13,2	13,6
37	13,5	14,8
41	13,7	15,0
45	12,9	13,9
49	13,8	13,8
53	14,0	14,1
57	13,8	13,9
61	14,0	fortgelaufen.
65	14,0	
67	14,1	
71	13,0	
74	13,3	
76	13,1	

## Versuch IV. (Taf. XXIV.)

## Geschälter Reis mit Roh-Oryzanin (I).

In diesem Versuche wurden 2 Mäuse (A) mit geschältem Reis und Roh-Oryzanin (I) gefüttert und zwei andere (B) wurden noch dazu mit Lecithin, Phytin und Salzen versehen. Alle blieben 35 Tage völlig gesund, bis der Versuch unterbrochen wurde. Man konnte jedoch zwischen A und B einen ziemlich grossen Unterschied merken, d. h. der Einfluss von Lecithin, Phytin und Salzen war deutlich erkennbar. Diejenigen, die keine Salze nebst Lecithin und Phytin bekommen haben (A), zeigten allmähliche Abnahme des Körpergewichts, während diejenigen, die sie bekommen haben (B), etwas an Körpergewicht zugenommen haben. Da der geschälte Reis sehr arm an Mineralstoffen ist und das Roh-Oryzanin vollkommen frei davon, so ist es wohl begreiflich, dass der Mangel an Mineralstoffen in diesem Falle (A) fühlbar war.

Die Futtergemische in diesem Versuche hatten folgende Zusammensetzung:

A. 100 g Reis und 0,2 g Roh-Oryzanin.

B. 100 g Reis, 0,2 g Roh-Oryzanin, 0,4 g Lecithin (Kahlbaum), 0,5 g Phytin, 0,3 g  $\text{CaCO}_3$ , 0,1 g  $\text{CaCl}_2$ , 0,2 g  $\text{Na}_2\text{CO}_3$ , 0,1 g  $\text{K}_2\text{CO}_3$ .

Aus oben angegebenen Beobachtungen ist also festgestellt worden, dass die Mäuse durch ausschliessliche Reisfütterung ohne Ausnahme binnen 10 bis 20 Tagen unter starker Abmagerung zugrunde gehen, während diejenigen, die entweder mit ungeschältem Reis oder mit geschältem Reis nebst Kleie oder alkoholischem Extrakt gefüttert werden, längere Zeit gesund und normal leben können.

Ganz dasselbe Resultat konnte man auch mit Roh-Oryzanin erzielen. Nur starben einige Tiere während der Versuche aus unbekannter Ursache. Dies kann aber selbst bei gewöhnlicher Fütterung oft geschehen.

Tabelle XXII.

Versuchstage	Körpergewicht und Nummer des Tieres			
	A		B	
	(1)	(2)	(1)	(2)
1	10,9	11,3	7,8	6,8
4	10,5	10,8	9,9	8,3
6	10,5	10,8	10,0	8,6
10	10,8	10,3	9,8	8,3
12	10,0	10,6	10,4	8,9
14	9,6	10,8	10,0	8,5
17	9,6	10,5	11,4	8,1
19	9,7	11,2	11,6	8,5
21	9,9	11,0	11,7	8,3
24	9,7	10,5	11,9	8,3
26	9,8	10,7	12,1	7,9
28	9,6	10,8	12,0	7,6
30	9,7	10,6	11,9	7,3
31	9,6	10,4	11,8	7,3
33	9,3	10,3	11,5	7,1
35	9,4	15,3	11,9	7,3

Die Zugabe von Salzen mit oder ohne Phytin, Lecithin und Phosphat scheint im allgemeinen einen günstigen Einfluss gehabt zu haben. Besonders günstig war das Calciumphosphat. Da aber, wie gesagt, die Individualität und das Alter der Tiere eine grosse Rolle dabei spielen, so kann man noch keinen bestimmten Schluss ziehen. Wir wollen später uns nochmals mit dieser Frage beschäftigen.

## Versuch V. (Taf. XXIV.)

## Hühnereier.

500 g geschälter Reis wurden mit 250 g frischen Eiern (Schale ausgenommen) vermischt, bei niedriger Temperatur getrocknet und 3 Mäusen gegeben. Alle 3 gingen zugrunde, und zwar das erste nach 9 Tagen, das zweite nach 23 Tagen und das dritte nach 35 Tagen. Das kleinste lebte am kürzesten, wohl wegen seiner Empfindlichkeit. Es scheint also, dass Hühnereier fast frei von Oryzanin sind.

Tabelle XXIII.

Versuchstage	Körpergewicht und Nummer des Tieres		
	(1)	(2)	(3)
1	5,4	5,9	9,8
3	5,7	6,0	9,6
5	5,8	6,3	9,4
7	5,0	5,9	8,9
9	starb	5,8	9,1
14		5,3	8,8
16		5,1	8,3
18		4,8	8,2
19		4,7	—
21		4,4	7,6
23		starb	8,1
25			8,6
26			8,3
29			7,5
31			6,7
33			6,6
35			6,5
—			starb

## Versuch VI. (Taf. XXIV.)

## Geschälter Reis mit Kuhmilch.

Es wurden zwei Versuche mit Kuhmilch ausgeführt.

I. Die Milch wurde bei niedriger Temperatur bis zum Trocknen eingedampft und mit Aether und dann mit heissem Alkohol dreimal extrahiert. 45 g des auf diese Weise gewonnenen Rückstandes wurden mit 90 g geschältem Reis vermischt und 4 Mäusen gegeben.

II. 109 g (=800 cem frischer Milch) der bei niedriger Temperatur getrockneten Milch wurden mit 218 g Reis vermischt und 4 Mäusen gegeben.

Im Versuche I gingen alle vier fast in derselben Zeit zugrunde, obgleich sie etwas länger als bei ausschliesslicher Reisfütterung lebten.

Im Versuche II starb eine in kurzer Zeit aus unbekannter Ursache. Die übrigen drei blieben 34 Tage vollkommen gesund und munter; die eine hat sogar bedeutend an Gewicht zugenommen, während zwei andere allmählich abgenommen haben.

Man sieht also, dass die Milch sich gegen Mäuse etwas anders verhielt als gegen Tauben. Auf Tauben hat die Milch nämlich keine günstige Wirkung gezeigt. Jedenfalls enthält die Milch irgendeinen Stoff<sup>1</sup>, der für Mäuse, nicht aber für Tauben günstig wirkt.

Tabelle XXIV.

( 1 )

Versuchstage	Körpergewicht und Nummer des Tieres			
	( 1 )	( 2 )	( 3 )	( 4 )
1	10,8	10,6	9,9	9,0
3	11,9	11,6	10,8	10,0
6	11,8	11,2	11,2	11,1

1. Man vergleiche die Arbeit von T. B. OSBORNE, The Role of different Proteins in Nutrition and Growth, Science N. S., Vol. 34. No. 882, p. 722—732 (24. Nov. 1911).

Versuchstage	Körpergewicht und Nummer des Tieres			
	(1)	(2)	(3)	(4)
8	11,8	11,4	11,4	11,4
10	11,1	11,5	11,0	11,0
12	10,1	10,4	11,4	10,4
14	10,0	9,9	9,6	9,5
16	9,2	8,9	8,5	9,0
18	8,7	8,5	7,9	8,5
20	8,4	8,0	7,5	8,1
22	7,7	7,0	7,0	7,6
24	7,0	starb	6,5	7,0
26	starb		6,0	starb
			starb	

( 2 )

Versuchstage	Körpergewicht und Nummer des Tieres			
	(1)	(3)	(3)	(4)
1	9,2	7,5	10,4	10,1
3	10,3	8,9	11,2	11,6
6	11,0	9,7	10,7	11,0
8	11,9	9,6	12,6	11,2
10	12,1	8,9	13,5	10,8
12	11,1	7,4	12,9	10,7
14	10,6	starb	12,6	11,3
16	10,7		12,9	11,4
18	11,2		13,0	11,1
20	11,2		14,0	11,1
22	11,4		14,1	11,2
24	11,1		15,0	10,7



Versuchstage	Körpergewicht und Nummer des Tieres			
	(1)	(2)	(3)	(4)
26	9,2		14,7	10,1
28	9,4		14,9	9,1
30	8,9		14,7	8,7
32	8,9		13,8	8,8
34	8,5		13,7	8,4

Versuch VII. (Taf. XXV.)

Geschälter Reis mit Margarine.

100 g Reis wurden mit 10 g Margarine vermischt und 2 Mäusen gegeben. Sie blieben nur etwas länger als bei ausschliesslicher Reisstütterung am Leben. Nach 22 bis 24 Tagen starben sie beide unter Abmagerung.

Tabelle XXV.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	17,9	15,9
3	18,2	16,6
5	17,9	16,8
7	17,6	16,5
9	16,7	16,0
11	16,1	14,2
13	15,9	14,4
15	15,7	14,1
17	14,0	13,0
19	—	—

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
21	12,3	12,0
22	12,2	11,6
23	starb	11,0 starb

## Versuch VIII. (Taf. XXV.)

Geschälter Reis mit alkoholischem Extrakt von Pferdefleisch.

500 g frisches Pferdefleisch wurden mit heissem 90% igem Alkohol wiederholt extrahiert. Der Extrakt wurde stark eingedampft und nochmals mit 90% igem Alkohol versetzt, von ungelöstem Rückstand abfiltriert um Fette und andere Verunreinigungen zu entfernen, und weiter eingengt. Der auf diese Weise erhaltene alkoholische Extrakt wurde mit 250 g geschältem Reis vermischt und 2 Mäusen gegeben. Sie starben nach 11 Tagen unter Abmagerung<sup>1</sup>.

Tabelle XXVI.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	21,5	20,4
3	21,9	20,3
5	22,1	20,2
7	22,7	19,5
9	18,5	18,7
11	19,5 starb	17,5 starb

1. Herr Dr. M. WATANABE im hiesigen Laboratorium hat später diesen Versuch wiederholt und viel bessere Resultate erzielt. Er konnte nämlich 2 Mäuse 80 Tage lang am Leben erhalten. So scheint, dass der alkoholische Extrakt des Pferdefleisches auch für Mäuse wirksam ist.

Versuch IX. (Taf. XXV.)

Hier wurde ein Futtergemisch mit möglichst reiner Stärke, Casein, Lecithin und Salzen, mit oder ohne Oryzanin zubereitet. Die Zusammensetzung desselben war wie folgt:

A. Ohne Oryzanin.

100 g Stärke, 10 g Casein, 0,5 g Lecithin, 0,4 g  $\text{CaCO}_3$ , 0,1 g  $\text{CaCl}_2$ , 0,2 g  $\text{Na}_2\text{CO}_3$ , 0,1 g  $\text{K}_2\text{CO}_3$ , 0,1 g  $\text{NaCl}$ , 0,1 g  $\text{MgSO}_4$ .

B. Mit Oryzanin.

Zu A wurde 0,2 g Roh-Oryzanin (I) zugegeben. Zur Bereitung des Futtergemisches wurde die Stärke vorher verkleistert und mit anderen Stoffen vermischt, bei niederer Temperatur getrocknet und in kleine Stückchen geschnitten.

Sämtliche Tiere in A und B starben binnen 39 Tagen. Der Unterschied war jedoch, dass die Tiere, die Oryzanin bekommen hatten (B), dreimal länger lebten als die (A), die kein Oryzanin bekamen, was bloss der Einwirkung des Oryzanins zuzuschreiben ist.

Dieser Versuch soll nochmals wiederholt werden. Die Stärke ohne Verkleisterung hätte vielleicht ein besseres Resultat erzielt. Auch die anorganischen Salze hatten keine richtige Zusammensetzung<sup>1</sup>.

1. Vor kurzem hat Herr Dr. M. WATANABE im hiesigen Laboratorium ein Salzgemisch nach RÖHMANN hergestellt, und zwar in folgendem Mengenverhältnis:

10 g phosphors. Kalk,	40 g saures phosphors. Kali,	20 g Natriumchlorid.
15 g Natriumcitrat,	8 g Magnesiumcitrat,	8 g Calciumlactat.

Er hat 20 g dieses Salzgemisches mit 1000 g geschältem Reis gemischt und Mäusen gegeben. Die Tiere lebten 28 Tage, während 2 Kontrolltiere ohne Salze in 14 bzw. 15 Tagen zugrunde gingen.

Auch bei Tauben hat dieses Salzgemisch eine günstige Wirkung gezeigt. Trotzdem konnte er niemals die Tiere ohne Oryzaninzugabe länger als 4 Wochen am Leben erhalten. Wird der alkoholische Extrakt der Kleie oder Roh-Oryzanin (I) nebst Röhmannschem Salzgemisch den Tauben oder Mäusen gegeben, so bleiben sie nicht nur beliebig lange am Leben, sondern nehmen bedeutend an Gewicht zu.

Vgl. RÖHMANN, Allg. med. Zentral-Ztg. 1903, Nr. 9.

Tabelle XXVII.

A. Ohne Oryzanin.

Versuchstage	Körpergewicht und Nummer des Tieres		
	(1)	(2)	(3)
1	11,1	8,7	9,2
3	10,3	7,1	8,2
5	9,9	6,4 starb	8,0
6	9,3		7,8
8	9,6		6,9
9	8,5		—
10	starb		9,6 starb

B. Mit Oryzanin.

Versuchstage	Körpergewicht und Nummer des Tieres		
	(1)	(2)	(3)
1	9,1	7,3	9,5
3	10,6	8,1	11,0
5	10,3	7,4	10,1
6	10,2	7,4	9,9
8	9,6	7,1	9,8
10	9,4	6,3	9,5
11	—	6,0 starb	—
12	—		—
13	9,4		10,0
15	9,5		9,2
17	8,8		9,9
20	8,5		10,1
22	8,4		10,5
24	9,4		10,7

Versuchstage	Körpergewicht und Nummer des Tieres		
	(1)	(2)	(3)
27	9,1		9,8
29	8,9		8,6
31	8,4		8,1 starb
33	7,9		
35	8,0		
37	7,6		
39	6,8 starb		

## D. Hunde.

## Versuch I.

Ein ausgewachsener Hund von mittlerer Grösse (6,47 kg) wurde mit gekochtem Reis und ausgekochtem Rückstand von Pferdefleisch nebst einer kleinen Menge Kochsalz gefüttert. Er frass am Anfang sehr gut. Nach 14 Tagen ging der Appetit beträchtlich zurück. Er wollte den Reis nicht und suchte nur nach Fleisch, und das Körpergewicht nahm nach und nach ab; nach 3 Wochen hatte er die Esslust vollständig verloren. Eine weitere Woche lebte er fast ausschliesslich von Wasser, bis er schliesslich abgemagert und ermattet war. Das Körpergewicht ging bis auf 5,8 kg herunter. Nach 2 bis 3 Tagen wäre er zweifellos zugrunde gegangen. Zu diesem Zeitpunkt, also am 29. Tage, wurden 3 g alkoholischer Extrakt der Kleie (=30 g Kleie) in wenig Wasser gelöst und per os gegeben. Schon am nächsten Tage kam etwas Esslust zurück und nach dem 3. Tage war der Appetit ganz normal. Das Körpergewicht hat auch allmählich zugenommen und nach 32 Tagen (d. h. am 60. Tage vom Anfang an) hat er 7,30 kg erreicht. Hier wurde die Zugabe des alkoholischen Extrakts eingestellt. Noch eine Woche hat das Körpergewicht immer zugenommen bis auf 7,9 kg und nachher ging es wieder allmählich zurück;

der Appetit hatte wieder nachgelassen. Der Hund frass nur Fleisch und schliesslich wollte er gar nichts mehr; nach 38 Tagen (d. h. am 98. Tage vom Anfang an) kam das Körpergewicht bis auf 6,10 kg herunter und das Tier war sehr ermattet. So wurden am nächsten Tage 0,4 g Roh-Oryzanin (1) per os gegeben. Diesmal war die Wirkung noch deutlicher als beim alkoholischen Extrakt: am 2. Tage hat er wieder normalen Appetit bekommen und wurde vollständig gesund und munter. Nach 20 Tagen (118 Tage vom Anfang an) stieg das Körpergewicht bis auf 7,9 kg. Hierauf wurde die Oryzaninzugabe eingestellt. Die Folge dessen war genau so wie die Erwartung. Der Hund verlor diesmal etwas schneller den Appetit als das erste Mal und nach 20 Tagen war er schon ermattet und das Körpergewicht ging bis auf 5,90 kg herunter. Jetzt wurden ihm anstatt Oryzanin ca. 3 g alkoholischer Extrakt<sup>1</sup> des Pferdefleisches (=aus 150 g frischem Fleisch) gegeben. Die Wirkung der letzteren war ebenso deutlich wie bei Oryzanin. Das Tier hat sich sehr rasch erholt und am 20. Tage erreichte es 7,5 kg. Nach Einstellung der Zugabe des alkoholischen Extrakts des Pferdefleisches war er wieder sehr schnell abgemagert und ermattet. Der Versuch wurde weiter fortgesetzt, immer mit demselben Resultate. (Vgl. Tabelle XXVIII und Taf. XXVI.)

## Versuch II.

Derselbe Versuch wurde nochmals mit einem kleineren ausgewachsenen Hunde (Körpergewicht 4,49 kg) wiederholt. Der Verlauf war genau derselbe wie beim ersten Hunde. Ohne Oryzaninzugabe konnte der Hund mit gekochtem Reis und ausgekochtem Rückstand des Pferdefleisches nicht existieren und am 28. Tage war er vollständig ermattet. Das Körpergewicht sank bis auf 3,7 kg. Hiernach bekam er 4 g alkoholischen Extrakt der Kleie. Er erholte sich ebenso rasch wie der erste Hund. Vom 42. Tage an hat er täglich 10 g Margarinebutter daneben bekommen.

1. 100 g frisches Pferdefleisch lieferten 2,1 g alkoholischen Extrakt, welcher 0,2244 g Gesamt-N, 0,0909 g Basen-N und 0,520 g Asche enthält. Eiweiss war nicht vorhanden.

Der Appetit wurde immer grösser. Am 60. Tage hat er das Gewicht von 4,9 kg erreicht. Hier wurde die Oryzaninzugabe eingestellt. Trotzdem hat er noch beinahe 3 Wochen nicht an Gewicht verloren; dann ging der Appetit allmählich zurück und am 98. Tage sank das Gewicht bis auf 3,8 kg herunter und das Tier ging schliesslich zugrunde. (Vgl. Tabelle XXVIII. und Taf. XXVI.)

### Versuch III.

Der dritte Versuch wurde mit einer Hündin ausgeführt, die noch 2 junge Hündchen säugte. Ohne Oryzanin war sie in 3 Wochen schon ermattet und gab keine Milch mehr. Hierauf wurde 0,4 g Roh-Oryzanin eingegeben. Die Wirkung desselben war ebensogut wie beim alkoholischen Extrakt selbst. Nach 20 Tagen (am 49. Tage vom Anfang an) hat sie wieder 6,6 kg erreicht. Nach Einstellung des Oryzanins hat sie beinahe 4 Wochen nichts an Gewicht verloren, dann ging es allmählich herunter. (Vgl. Tabelle XXIX und Taf. XXVI.)

Tabelle XXVIII.

	Versuchs- tage	Körpergewicht		Bemerkungen
		(A)	(B)	
(A) und (B) ohne Oryzanin	1	6,47	4,49	(A) (B) Gesund
	2	6,40	4,60	
	3	6,60	4,60	
	4	6,30	4,40	
	5	6,20	4,40	
	6	6,20	4,50	
	7	—	—	
	8	6,80	4,70	Allmählich Appetit verloren
	9	6,80	4,70	
	10	6,70	4,70	
	11	6,70	4,50	

	Versuchs- tage	Körpergewicht		Bemerkungen
		(A)	(B)	
(A) und (B) ohne Oryzanin	12	6,80	4,70	
	13	6,80	4,70	
	14	6,80	4,80	
	15	6,90	4,70	
	16	6,80	4,70	
	17	6,80	4,80	
	18	6,50	4,60	
	19	6,40	4,50	
	20	6,40	4,50	
	21	6,20	4,20	Frisst nur Fleisch; (A) Er- brechen, Faeces weich
	22	6,20	4,20	
	23	6,10	4,10	(A) (B) Nichts gefressen
	24	6,00	4,20	Ermattet
	25	5,90	4,10	
(A) und (B) mit alkoholischem Extrakt der Kleie	26	5,80	4,20	
	27	5,70	4,10	
	28	5,60	3,80	Schwach
	29	5,60	3,80	(A) (B) 3 g alk. Extrakt per os gegeben
	30	5,50	3,70	Wieder Appetit
	31	6,20	4,30	Viel gefressen
	32	6,40	4,20	
	33	6,30	4,20	
	34	6,30	4,20	
	35	6,30	4,20	
	36	6,45	4,20	
	37	6,45	4,20	
	38	6,30	4,10	(A) Etwas appetitlos
	39	6,00	4,20	
	40	6,00	4,20	



	Versuchstage	Körpergewicht		Bemerkungen
		( A )	( B )	
(A) und (B) mit alkoholischem Extrakt der Kleie	41	6,50	4,35	(A) wieder Appetit bekommen
	42	6,70	4,20	(B) bekommt täglich 10 g Margarine
	43	6,65	4,15	
	44	6,75	4,10	
	45	6,50	4,15	(A) vollkommen gesund
	46	6,40	4,00	
	47	6,55	4,00	
	48	6,75	4,20	
	49	6,75	4,30	
	50	6,90	4,40	
	51	6,90	4,50	
	52	7,05	4,60	
	53	7,00	4,60	
	54	6,90	4,55	
	55	7,00	4,50	
	56	7,00	4,60	
	57	7,20	4,70	
	58	7,10	4,80	
	59	7,50	4,90	(A) (B) Vollständig gesund
	60	7,30	4,90	
(A) und (B) ohne Oryzanin	61	7,40	4,70	(A) (B) Alkoholextrakt eingestellt
	62	7,50	4,70	
	63	7,50	4,65	
	64	7,60	4,90	
	65	7,60	4,80	
	66	7,80	5,00	
	67	7,90	5,00	
	68	7,90	4,90	
	69	7,90	4,95	

	Versuchs- tage	Körpergewicht		Bemerkungen
		( A )	( B )	
(A) und (B) ohne Oryzanin	70	7,80	4,90	(A) Appetit zurück
	71	7,70	5,00	
	72	7,60	5,00	
	73	7,60	5,00	
	74	7,40	4,90	
	75	7,35	4,95	
	76	7,30	5,00	(B) Appetit zurück
	77	7,30	4,90	
	78	7,05	4,95	
	79	7,10	4,90	
	80	6,90	4,90	
	81	6,80	4,70	
	82	6,70	4,60	(B) Frass nur Fleisch
	83	6,60	4,65	
	84	6,80	4,75	
	85	6,70	4,55	
	86	6,60	4,50	
	87	6,60	4,40	
	88	6,60	4,30	(A) Frass nur wenig Fleisch
	89	6,70	4,40	
	90	6,60	4,40	
	91	6,45	4,35	(B) Appetitlos
	92	6,40	4,20	(A) Nichts gefressen
	93	—	4,30	
	94	6,35	4,25	(B) Schwach; Erbrechen
	95	6,30	4,20	
	96	6,25	4,10	(B) Sehr schwach
	97	6,15	4,00	
	98	6,15	3,80	(A) Schwach

	Versuchs- tage	Körpergewicht		Bemerkungen
		( A )	( B )	
(A) 0,4 g Roh-Oryzanin (1) täglich	99	6,10	storb	(A) 0,4 g Roh-Oryzanin (1) per os
	100	6,25		
	101	6,90		Appetit kommt wieder
	102	6,95		Gesund
	103	7,00		
	104	7,10		
	105	7,20		
	106	7,20		
	107	7,30		
	108	7,40		
	109	7,30		
	110	7,50		
	111	7,40		
	112	7,50		
	113	7,70		
	114	7,60		
	115	7,80		
	116	7,80		
	117	7,75		
	118	7,90		Vollständig gesund
(A) Ohne Oryzanin	119	7,80		Oryzanin eingestellt
	120	7,80		
	121	7,80		
	122	7,70		
	123	7,60		
	124	7,50		
	125	7,40		
	126	7,20		
	127	7,10		

	Versuchstage	Körpergewicht		Bemerkungen
		(A)	(B)	
(A) ohne Oryzanin	128	7,00		
	129	6,90		
	120	6,80		
	121	6,70		
	132	6,60		
	133	6,45		
	134	6,30		
	135	6,30		
	136	6,20		
	137	6,05		
	138	5,90		
Alkoholischer Extrakt aus 150 g Pferdefleisch täglich	139	5,90		
	140	6,10		
	141	6,30		
	142	6,70		
	143	6,50		
	144	6,80		
	145	6,90		
	146	7,00		
	147	7,00		
	148	6,70		
	149	7,00		
	150	7,10		
	151	6,90		
	152	6,90		
	153	7,00		
	154	7,20		
	155	7,40		
	156	7,40		

	Versuchstage	Körpergewicht		Bemerkungen
		(A)	(B)	
Alkoholischer Extrakt aus 150 g Pferdefleisch täglich	157	7,40		Gesund und munter
	158	7,50		
Ohne Oryzanin	159	7,50		
	160	7,40		
	161	7,20		
	162	7,20		
	163	7,20		
	164	7,20		
	165	7,00		
	166	7,00		
	167	7,00		
	168	6,90		
	169	6,70		Esslust nachgelassen
	170	6,60		
	171	6,50		
	172	6,40		
	173	6,30		
	174*	6,10		
	175*	6,00		
	176	6,00		
	177	5,80		
	178**	5,80		
	179†	6,00		* Durch Phosphorwolframsäure fällbarer Teil des alkoholischen Extrakts aus 250 g Pferdefleisch. Keine Wirkung.  ** Filtrat von Phosphorwolframsäure Niederschlag aus 500 g Pferdefleisch. keine Wirkung und Erbrechen; so wurde der Alkoholextrakt aus †) 200 g Kleie und am nächsten Tage derselbe aus 100 g Kleie gegeben. Das Tier wieder geheilt und hat Appetit bekommen.
	180	6,10		
	181	6,20		
	182	6,20		
	183	6,40		
	184	6,60		
	185	6,80		

	Versuchs- tage	Körpergewicht		Bemerkungen
		( A )	( B )	
Ohne Oryzanin	186	6,70		
	187	6,80		
	188	6,90		
	189	—		
	190	6,60		
	191	—		
	192	7,60		
	193	6,50		
	194	6,80		
	195	6,50		
	196	6,30		
	197	6,30		
	198	6,20		
	199	6,10		
	200	—		
	201	6,60		Etwas gefressen
	202	6,40		
	203	6,20		
	204	6,20		
	205	6,20		
	206	6,10		
	207	6,30		
	208	6,40		
	209	6,60		Etwas gefressen
	210	6,60		
	211	6,10		
	212	6,40		
	213	6,10		
	214	6,30		

	Versuchstage	Körpergewicht		Bemerkungen
		( A )	( B )	
Ohne Oryzanin	215	6,30		Allmählich schwach
	216	6,50		
	217	6,40		Appetitlos
	218	6,30		Nichts gefressen
	219	6,10		
	220	6,00		
	221	6,20		
	222	6,00		
	223	5,80		Enmattet
				Versuch fortgesetzt
				0,5 g Alkoholextrakt der Kleie täglich gegeben; wieder geheilt und nahm an Gewicht zu

Tabelle XXIX.

	Versuchstage	Körpergewicht	Bemerkungen
		( C )	
Reis + ausgekochtes Pferdefleisch	1	7,00	Gesund
	3	6,90	
	5	6,70	
	7	6,50	
	9	6,60	
	11	6,20	Appetit nachgelassen
	13	6,20	
	15	6,10	Schwach
	17	5,80	
	19	5,60	Enmattet

	Versuchstage	Körpergewicht	Bemerkungen
		(C)	
0,4 g Roh-Oryzanin (I) täglich	20	5,50	Geheilt; Appetit bekommen
	21	5,65	
	22	5,70	
	23	5,75	Gesund und munter
	25	5,90	
	27	5,80	
	29	6,20	
	31	6,30	
	33	6,60	
	35	6,70	
	37	6,60	
	39	6,60	
	41	6,60	
	43	6,60	
	45	6,60	
	47	6,60	
	49	6,60	Gesund
Ohne Oryzanin	51	6,70	* Herausgekommen und etwas Schweinefleisch gefressen
	53	6,40	
	55	6,50	
	57	6,50	
	59	6,40	
	61*	6,50	
	63	6,60	** Wieder herausgekommen
	65	6,60	
	67**	6,60	
	69	6,55	
	71	6,60	
	73	6,40	



	Versuchstage	Körpergewicht	Bemerkungen
		(C)	
Ohne Oryzanin	75	6,60	
	77	6,40	
	79	6,50	
	81	6,50	
	83	6,30	
	85	6,50	
	87	6,40	
	89	—	
	91	6,50	
	93	6,30	
	95	6,20	
	99	6,20	
	101	6,00	
	103	6,00	
	105	6,00	Allmählich schwach
	107	6,10	Appetitlos
	109	6,30	
	111	6,00	
	113	6,10	
	115	6,20	
	117	5,90	Nichts gefressen
	119	5,70	
	120	5,10	Ermattet; 0,5 g Alkoholextrakt der Kleie
			Versuch fortgesetzt
			Mit 0,5 g Alkoholextrakt der Kleie wieder geheilt und nahm an Gewicht zu.

Das Futtergemisch hatte folgende Zusammensetzung:

500 g geschälter Reis,

120 g ausgekochter Rückstand von Pferdefleisch<sup>1</sup>,

5 g Kochsalz,

5 g Salzgemisch ( $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$ ,  $\text{MgCO}_3$ ,  $\text{CaCO}_3$ ).

Aus diesem Resultat sieht man also, dass das Leben des Hundes vollständig unter Kontrolle des alkoholischen Extrakts der Kleie bzw. des Oryzanins steht. Die Wirkung des alkoholischen Extrakts aus Pferdefleisch war ebenso gut wie beim Oryzanin. Da der alkoholische Extrakt aus Fleisch auf Tauben und Hühner fast wirkungslos war, so muss man annehmen, dass der wirksame Stoff des Fleisches mit dem Oryzanin nicht identisch ist. Beim Hunde (und bei Mäusen) kann der eine den anderen vertreten, während bei Tauben und Hühnern dies nicht der Fall ist.

Wir haben schon einige Versuche angestellt, um diesen wirksamen Stoff im Fleische ausfindig zu machen. Wenn der alkoholische Extrakt des Fleisches in Wasser gelöst und mit Bleiessig gefällt wird, so findet man im Filtrate des Niederschlages keine Wirkung mehr. Man muss deshalb annehmen, dass der wirksame Stoff entweder durch Bleiessig mitgerissen wird oder während der Verarbeitung zerstört wird. Wir wollen uns mit dieser Frage weiter beschäftigen.

#### IV. Ueber die Verbreitung des Oryzanins in verschiedenen Futtermitteln.

Da wir noch keine zuverlässige Bestimmungsmethode des Oryzanins gefunden haben, so bleibt uns nichts übrig, als durch Tierversuche die

1. Das frische Fleisch wurde zweimal je eine Stunde mit Wasser gekocht und stark abgepresst, bis keine löslichen Stoffe mehr vorhanden waren. Der so erhaltene Rückstand enthielt:

Wasser	46,56%,
Trockensubstanz	53,44%.
In 100 Teilen Trockensubstanz;	
Gesamt-N	15,02%,
Rohfette	3,51%,
Asche	0,48%.

Verbreitung und annähernde Quantität desselben in verschiedenen Futtermitteln festzustellen. Zu diesem Zwecke haben wir hauptsächlich Tauben und Mäuse benutzt. Die Tiere werden so lange mit geschältem Reis gefüttert, bis sie schliesslich krank werden, hierauf gibt man den alkoholischen Extrakt der verschiedenen Futtermittel per so oder mit Reis gemischt und beobachtet, ob sie geheilt werden oder nicht. Oder man gibt von Anfang an den alkoholischen Extrakt der betreffenden Futtermittel mit Reis gemischt an gesunde Tiere, um zu sehen, ob sie schliesslich krank werden.

Diejenigen Stoffe, die die Tiere gern fressen, haben wir als solche gegeben, z. B. wie Gerste, Weizen, Brot usw. In folgenden teilen wir einige Versuche in dieser Richtung mit.

### Versuch I.

#### Weizenkleie.

1. Die käufliche Weizenkleie wurde genau so wie Reiskleie wiederholt mit 85% igem Alkohol heiss extrahiert. Der alkoholische Auszug wurde abdestilliert. Der zurückgebliebene Sirup wurde mit wenig Wasser verdünnt und wiederholt mit Aether geschüttelt, der Aether verdampft und abfiltriert. Der in der Weise aus 500 g Weizenkleie dargestellte alkoholische Extrakt wurde mit 500 g Reis gemischt, getrocknet und einer gesunden Tauben gegeben. Die Taube blieb 16 Tage vollkommen gesund und nahm sogar etwas an Körpergewicht zu.

Tabelle XXX.

Versuchstage	Körpergewicht
1	300
3	308
5	314
7	321

Versuchstage	Körpergewicht
9	331
11	330
13	334
15	335
17	336

2. Wenn der alkoholische Extrakt aus 300 g Weizenkleie mit 1000 g Reis vermischt wird, so genügt dies noch nicht, um eine Taube vor der Erkrankung zu schützen, und das Tier nimmt an Gewicht ab. Wenn aber täglich ein aus 10 g Weizenkleie bereiteter alkoholischer Extrakt noch dazugegeben wird, so wird das Tier sehr rasch geheilt und nimmt an Gewicht zu.

3. Der alkoholische Extrakt der Weizenkleie wurde in wenig Wasser gelöst und unmittelbar mit Tannin gefällt. Der Tanninniederschlag wurde in gewöhnlicher Weise in verdünntem Aceton gelöst, mit Baryt zerlegt und genau so verarbeitet, wie bei der Reiskleie. 0,09 g des so bereiteten Roh-Oryzanins gab ein positives Resultat.

4. Das aus dem alkoholischen Extrakt der Weizenkleie durch das Phosphorwolframsäureverfahren dargestellte Roh-Oryzanin war auch wirksam. Man musste jedoch täglich aus 60 g Kleie dargestelltes Roh-Oryzanin verwenden, um eine Taube zu heilen.

Tabelle XXXI.

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	{	310	Gesund
		...	
		221	
		223	Erkrankt
		217	

	Versuchstage	Körpergewicht	Bemerkungen
0,09 g Roh-Oryzanin (Tanninverfahren) aus Weizenkleie	1	213	Geheilt
	3	219	
	5	220	
	7	223	
	9	224	
	11	225	
	13	232	
	15	243	
	17	238	
	19	244	
Reis allein	21	248	Gesund
	23	246	
	25	251	
	27	251	
	28	248	
			Noch nicht erkrankt

Tabelle XXXII.

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein		270	Gesund
		231	Erkrankt
Roh-Oryzanin(Phosphor- wolframsäureverfahren) aus 60 g Kleie	1	215	Geheilt
	2	225	
	3	225	
	4	226	
	5	229	

	Versuchstage	Körpergewicht	Bemerkungen
Roh-Oryzanin(Phosphor- wolframsäureverfahren) aus 60 g Kleie	6	223	
	7	227	
	8	230	
	9	225	Gesund

Aus dem oben angegebenen Resultate kann man schliessen, dass der Gehalt an Oryzanin in der Weizenkleie etwa  $\frac{1}{10}$  des der Reiskleie ist.

Während der Verarbeitung des Phosphorwolframsäureniederschlages haben wir ziemlich viel Betain gefunden. Wenn der Niederschlag in gewöhnlicher Weise durch Baryt zerlegt und der Ueberschuss von Baryt mittels Schwefelsäure genau entfernt wird, so erhält man eine schwach alkalisch reagierende Flüssigkeit, die neben Oryzanin ziemlich viel Betain enthält. Wird nun diese Flüssigkeit stark eingeeengt und mit Aethylalkohol und Aceton versetzt, so scheidet sich ein wenig eines anorganischen Salzes ab. Man filtriert davon ab, dampft das Filtrat weiter ein und lässt einige Tage stehen, so scheiden sich die charakteristischen Krystalle des Betains als hygroskopische, farblose Tafeln aus; es hat einen angenehmen, süssen Geschmack und zersetzt sich bei ca.  $250^{\circ}$ , ohne zu schmelzen.

Die Ausbeute an freiem Betain beträgt ca. 6 g aus 1 kg Weizenkleie. Wird die wässrige Lösung des Betains mit Pikrinsäure versetzt, so erhält man das Betainpikrat als citronengelbe Prismen. Einmal aus heissem Wasser umkrystallisiert, schmilzt es bei  $180^{\circ}$  (unkorr.).

Die Analyse des Pikrats gab folgende Resultate:

1.	0,1510 g	Subst.	gaben	0,2116 g	CO <sub>2</sub> ,	0,0602 g	H <sub>2</sub> O.
2.	0,1130 "	"	"	0,1582 "	"	0,0482 "	"
3.	0,0912 "	"	"	13,4 ccm	N (21,5°	757,5 mm).	
4.	0,2776 "	"	"	0,1856 g	Pikrinsäure.		
				C	H	N	Pikrinsäure
C <sub>2</sub> H <sub>11</sub> ,NO <sub>2</sub> C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub> ...				Ber. 38,15	4,05	16,18	66,19
				Gef. 38,21	4,43	16,62	66,86
				38,17	4,74	—	—

## Weizenbrot.

Das gewöhnliche Weizenbrot wurde zerkleinert und an zwei Tauben verfüttert. 100 Tage lang blieben sie gesund und normal.

Tabelle XXXIII.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	303	300
5	300	260
10	290	270
15	295	285
20	295	280
25	300	298
30	305	307
35	310	328
40	310	332
45	300	325
50	290	308
55	300	324
60	300	310
65	290	300
70	298	310
75	295	310
80	296	300
85	290	285
90	285	282
95	297	295
100	283	296

## Versuch II.

## Gerstenkleie.

Wenn man den alkoholischen Extrakt aus 15 g Gerstenkleie täglich einer erkrankten Taube gibt, so wird die Taube sehr rasch geheilt, wie folgende Versuche zeigen.

Wie diese Tabelle zeigt, haben die beiden erkrankten Tauben nach 6 tägigem Zusatz vom alkoholischen Extrakt aus 15 g Gerstenkleie sich vollständig erholt und sind später noch 10 Tage lang ohne alkoholischen Extrakt vollständig gesund geblieben. So kann man berechnen, dass das Oryzanin der Gerstenkleie wenigstens  $\frac{1}{5}$  des der Reiskleie ist. Wir konnten jedoch kein wirksames Präparat, weder durch Phosphorwolframsäure noch durch das Tanninverfahren bekommen.

Tabelle XXXIV.

	Versuchstage	Körpergewicht und Nummer des Tieres		Bemerkungen
		(1)	(2)	
Reis allein		296	310	Gesund
		...	...	
		212	191	Erkrankt
		207	190	
Alkoholischer Extrakt aus 15 g Gerstenkleie täglich	1	208	188	Geheilt
	2	222	199	
	3	220	193	Gesund
	4	233	201	
	5	237	203	
	6	244	207	
Reis allein	7	247	209	
	8	256	210	
	9	257	220	



	Versuchs- tage	Körpergewicht und Nummer des Tieres		Bemerkungen
		(1)	(2)	
Reis allein	10	254	221	
	11	251	222	
	12	249	222	
	13	245	222	
	14	243	227	
	15	236	223	
				Noch nicht erkrankt

### Versuch III.

#### Hafer.

Der alkoholische Extrakt aus 1 kg Hafer wurde mit 1 kg Reis vermischt, getrocknet und zwei erkrankten Tauben gegeben. Die eine war jedoch schon zu schwach, um genug Futter zu fressen und ging schliesslich zugrunde; die andere aber erholte sich allmählich und wurde später vollständig gesund.

Der Gehalt von Oryzanin im Hafer muss deshalb ungefähr  $\frac{1}{10}$  des der Reiskleie sein.

Tabelle XXXV.

Versuchstage	Körpergewicht und Nummer des Tieres		Bemerkungen
	(1)	(2)	
	...	...	
1	285	227	1, 2 erkrankt
3	294	228	
5	288	221	
7	278	220	
9	267	217	
11	255	212	

Versuchstage	Körpergewicht und Nummer des Tieres		Bemerkungen
	(1)	(2)	
13	245	225	2 geheilt
15	239	230	
17	starb	228	
19		228	
21		233	
23		238	
25		243	2 gesund
27		245	

## Versuch IV.

## Hirse.

Der alkoholische Extrakt aus 50 g Hirse täglich war genügend, um eine erkrankte Taube zu heilen.

Tabelle XXXVI.

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein		286	Gesund
		249	
		246	
Alkoholischer Extrakt aus 50 g Hirse	1	246	Geheilt
	3	250	
	5	257	
	7	264	
	9	270	
	10	273	Gesund

	Versuchstage	Körpergewicht	Bemerkungen
Reis allein	12	274	Gesund
	14	280	
	16	275	
	18	274	
	20	265	
	22	261	
	24	250	Allmählich schwach
	25	246	

Versuch V.

*Brassica* var. (Kyona).

400 g der lufttrockenen Blätter und Stengel von *Brassica* var. wurden mit heissem 85% igem Alkohol wiederholt extrahiert. Der alkoholische Extrakt wurde mit 400 g Reis vermischt, getrocknet und einer Taube eingegeben. Die Taube hat 17 Tage lang nichts an Körpergewicht verloren. Nach 18 Tagen wurde der alkoholische Extrakt auf die Hälfte reduziert. Nun nahm das Körpergewicht allmählich ab und schliesslich erkrankte das Tier.

Tabelle XXXVII.

	Versuchstage	Körpergewicht	Bemerkungen
Alkoholischer Extrakt aus 400 g <i>Brassica</i> var. auf 400 g Reis	1	235	Gesund
	3	250	
	5	243	
	7	237	
	9	248	

	Versuchstage	Körpergewicht	Bemerkungen
Alkoholischer Extrakt aus 400 g Brassica var. auf 400 g Reis	11	260	Gesund
	13	256	
	15	260	
	17	268	
Alkoholischer Extrakt aus 200 g Brassica auf 400 g Reis	19	262	Schwach Erkrankt
	21	258	
	23	257	
	25	250	
	27	246	
	29	239	
	31	234	
	33	232	
	34	227	

Der Gehalt an Oryzanin in lufttrockener Brassica muss also ungefähr  $\frac{1}{10}$  des der Reiskleie sein.

Weder durch Phosphorwolframsäure noch durch Tannin konnten wir ein wirksames Präparat erhalten.

#### Versuch VI.

##### Hühnereier.

250 g frische Hühnereier (Schale ausgenommen) wurden mit 500 g Reis vermischt, getrocknet und an zwei Tauben verfüttert.

Nach 2 Wochen waren die beiden Tauben stark ermattet und erkrankt. Hierauf wurde der alkoholische Extrakt der Weizenkleie per os gegeben; sie wurden sehr rasch geheilt und das Körpergewicht hatte auch stark zugenommen. Dass die Eier keine schädlichen Bestandteile für die Tauben in sich enthalten, sondern dass die Erkrankung bloss durch Mangel an Oryzanin hervorgerufen ist, geht ganz klar aus diesem Versuch hervor.

Tabelle XXXVIII.

	Versuchs- tage	Körpergewicht und Nummer des Thieres		Bemerkungen
		( 1 )	( 2 )	
Reis+Hühnereier	1	242	242	Gesund
	2	256	256	
	3	262	278	
	4	265	284	
	5	265	287	
	6	260	289	
	7	257	280	
	8	245	267	
	9	237	250	
	10	221	246	
	11	217	235	
	12	210	227	
	13	205	219	
	14	215	214	
Reis+Hühnereier +alkoholisch. Extrakt aus Weizenkleie	15	203	206	Geheilt
	16	210	223	
	17	227	229	
	18	238	232	
	19	247	243	
	20	256	248	
	21	257	257	
	22	258	260	
				Gesund

## Versuch VII.

## Kuhmilch.

Wenn man die Taube mit Reis und alkoholischem Extrakt von Kuhmilch füttert, so lebt das Tier etwas länger als mit Reis allein, trotzdem verliert es allmählich an Körpergewicht und wird schliesslich krank. Auch frische Milch wirkt kaum bessers als der alkoholische Extrakt. Wird das Oryzanin in diesem Falle der erkrankten Taube gegeben, so wird das Tier in einigen Tagen geheilt, oder wenn dass Oryzanin von Anfang an mit Milch zusammengegeben wird, so bleibt das Tier längere Zeit gesund und normal. Hieraus muss man schliessen, dass der Milch nur Oryzanin fehlt. Etwas anders verhält sich die Milch gegen Mäuse<sup>1</sup>. Das letztgenannte Tier kann natürlich längere Zeit von Reis und Milch leben, wie auf den folgenden Seiten beschrieben wird.

## Versuch VIII.

## Adzukibohnen.

1. Der alkoholische Extrakt aus 300 g Adzukibohnen wurde mit 100 g Reis vermischt und an zwei Tauben verfüttert. Sie erkrankten ebenso schnell wie mit Reis allein. In 12 Tagen hat eine Taube von 296 bis 231 g und die andere von 287 bis 223 g abgenommen und beide waren sehr geschwächt. Hierauf wurde der einen Taube der alkoholische Extrakt aus 9 g Adzukibohnen per os gegeben, ohne günstige Wirkung zu zeigen.

2. In diesem Versuche wurden zwei Tauben ausschliesslich mit zerkleinerten Bohnen gefüttert. Sie frassen täglich 20 bis 25 g Bohnen und blieben längere Zeit gesund. Das Körpergewicht nahm auch allmählich zu.

1. P. E. OSBORNE, Science N. S. 34, Nr. 882, S. 722 bis 732 (24. Nov. 1911).

Tabelle XXXIX.

Versuchstage	Körpergewicht und Nummer des Thieres	
	( 1 )	( 2 )
1	304	301
3	288	285
5	295	300
7	294	304
9	298	302
11	295	305
13	300	300
15	308	303
17	314	312
19	310	308
21	306	300
23	307	305
25	310	312
27	315	312
29	317	312
31	313	313
33	315	315
35	311	308
37	315	315
37	318	320
41	321	312
43	315	320
45	—	—
47	305	315
49	325	330
51	315	320
53	317	321

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
55	317	322
57	299	298
59	311	321
61	313	326
63	306	318
65	315	332
67	310	325

Demnach ist der Gehalt an Oryzanin in Adzukibohnen höchstens  $\frac{1}{10}$  des der Reiskleie.

#### Versuch IX.

##### Sojabohnen.

1. Der alkoholische Extrakt aus 300 g Sojabohnen wurde mit 1000 g Reis vermischt und zwei gesunden Tauben gegeben. Auch in diesem Falle wurde keine Schutzwirkung beobachtet. Die eine hatte von 307 bis 241 g und die andere von 318 bis 258 g abgenommen.

2. Wenn man aber den Tauben nicht den alkoholischen Extrakt der Sojabohnen, sondern die Bohnen selbst gibt, so bleiben die Tiere längere Zeit gesund.

Demnach muss man annehmen, das in Sojabohnen nicht viel Oryzanin vorhanden ist.



Tabelle XL.

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
1	308	303
3	310	315
5	310	323
7	309	—
9	304	—
11	305	—
13	314	—
15	316	—
17	320	318
19	320	322
21	322	325
23	317	327
25	325	336
27	320	331
29	330	343
31	327	332
33	335	340
35	327	337
37	325	333
39	330	330
41	330	338
43	326	332
45	320	329
47	330	320
49	324	320
51	328	318
53	321	320

Versuchstage	Körpergewicht und Nummer des Tieres	
	(1)	(2)
55	—	—
57	324	307
59	321	303
61	325	323
63	322	323
95	328	322
67	331	323
69	325	323
71	324	320

## Versuch X.

## Entkleiete Gerste.

1. Känflche entkleiete Gerste, die zum Kochen fertig ist, wurde an 2 Tauben verfüttert, ohne dass dieselben längere Zeit hindurch krank wurden. Weitere 2 Tauben wurden mit in Wasser gekochter Gerste gefüttert; sie blieben auch längere Zeit gesund. So scheint es, dass beim Entkleien und Kochen noch eine genügende Menge Oryzanin zurückbleibt, um die Tauben vor Erkrankung zu schützen.

Tabelle XLI.

Versuchstage	Körpergewicht und Nummer des Tieres			
	Entkleiete Gerste		Dieselbe mit Wasser gekocht	
	(1)	(2)	(3)	(4)
1	270	280	275	310
5	256	295	282	302
10	268	302	279	297
15	272	305	272	295
20	297	315	300	310
25	282	317	292	308
30	276	317	313	328
35	260	302	310	310
40	270	315	316	323
45	265	320	312	315
50	263	312	318	314
55	245	320	325	324
60	230	318	325	340
65	220	325	320	335
70	230	323	318	313
75	215	321	323	330
80	230	330	306	303
85	Entflohen	333	320	323

Bei Gerste ist die Kleieschicht nicht so scharf von dem inneren Teil getrennt wie beim Reis, so dass beim Polieren immer noch etwas Schale zurückbleibt. Wahrscheinlich findet sich im Kern selbst auch etwas Oryzanin.

## Versuch XI.

## Gerstenmalz.

Das Brauermalz, das wir von Herrn Braumeister Dr. Jagi aus der Sapporo-Brauerei bekomme haben, wurde fein zerkleinert und mit heissem Alkohol wiederholt extrahiert. Die alkoholischen Auszüge wurden nach dem Verdampfen des Alkohols in wenig Wasser gelöst und wiederholt mit Aether geschüttelt, klar abfiltriert und weiter konzentriert. Der auf diese Weise dargestellte alkoholische Extrakt hat sich als wirksam erwiesen. Man musste jedoch ziemlich grosse Mengen desselben anwenden. Der Extrakt aus 50 g Malz war kaum genug, um eine erkrankte Taube zu heilen. Derselbe aus 75 g reichte schon aus, um das Tier längere Zeit im Gleichgewicht zu halten.

Tabelle XLII.

	Versuchstage	Körpergewicht	Bemerkungen
Re's allein		⋮	Gesund
		247	
		214	Erkrankt
Alkoholischer Extrakt aus 50 g Malz täglich	1	202	Geheilt
	2	217	
	3	213	
	4	212	
	5	212	
	6	215	Gesund

	Versuchstage	Körpergewicht	Bemerkungen
Derselbe aus 75 g Malz	7	210	
	8	207	
	9	215	
	10	213	
	11	218	
	12	219	
	13	219	
	14	212	
	15	221	
	16	218	
	17	218	Gesund
Reis allein	19	233	
	21	223	
	23	230	
	25	235	
	27	228	Gesund
	29	220	
	31	209	
	33	198	Erkrankt

## Versuch XII.

## Bier.

Das „Münchener“ Bier aus der Yebisu-Brauerei wurde bei gelinder Wärme zu einem Sirup eingedampft und mit heissem 90% igem Alkohol extrahiert. Der alkoholische Extrakt wurde wieder eingedampft und nochmals mit heissem Alkohol extrahiert. Der in dieser Weise aus 500 cem Bier gewonnene Extrakt wurde täglich einer erkrankten Taube gegeben, hatte aber keine Schutzwirkung.

Es scheint also, dass das Oryzanin im Malz während des Gärprozesses verloren gegangen ist.

### Versuch XIII.

Gewöhnliche Möhre: Ninjin (*Daucus carota*).

Der alkoholische Extrakt aus 100 g frischer Möhren hatte keine Schutzwirkung. Auch konnten wir durch das Tanninverfahren kein wirksames Präparat bekommen. Ob wirklich Oryzanin darin fehlt, muss durch weitere Versuche noch festgestellt werden, weil Kaninchen entweder mit Möhren allein oder mit Möhren und Reis gefüttert längere Zeit gesund geblieben sind.

### Versuch XIV.

Raphanusblätter (Daikon).

Das aus 100 g lufttrockenen Raphanusblättern durch Tanninverfahren dargestellte Roh-Oryzanin hatte Schutzwirkung, obgleich diese nicht sehr stark war.

### Versuch XV.

Miso und Schoyu.

Da Miso sehr stark salzhaltig ist, kann man sie natürlich nicht direkt an Tauben geben, auch dessen alkoholischer Extrakt enthält eine beträchtliche Menge Salze und Extraktivstoffe; so haben wir mittels des Phosphorwolframsäureverfahrens aus alkoholischem Extrakt ein Roh-Oryzaninpräparat dargestellt. Die Wirkung desselben war jedoch nur unbedeutend.

Vielleicht kann man am Hund oder an der Katze, die gerne Miso fressen, noch besser entscheidende Resultate bekommen. Es bleibt nur die Frage, ob man mit grösserer Menge ein etwas wirksames Präparat bekommen kann. Jedenfalls ist es sicher, dass sowohl Miso wie Schoyu keine nennenswerte Menge Oryzanin enthalten.

## V. Zusammenfassung der Resultate.

1. Hühner, Tauben, Mäuse und einige andere Tiere werden durch ausschliessliches Füttern mit geschältem Reis leicht krank und gehen unter starker Abnahme des Körpergewichts zugrunde. Diese Erscheinung ist durch Mangel an einem Stoffe im Reis, der für die Erhaltung des tierischen Lebens absolut notwendig ist, bedingt.

2. Dieser unentbehrliche Stoff ist nun aus Reiskleie in reinem Zustande isoliert worden. Wir haben für diesen Stoff den Namen „Oryzanin“ vorgeschlagen.

Das Oryzanin nimmt eine ganz besondere und ebenso wichtige Stellung im Haushalte des tierischen Lebens ein, wie Eiweiss, Fett, Kohlenhydrate und Salze. Ohne diese können die letztgenannten Stoffe keine physiologische Funktion entfalten.

3. Jedes Futtermittel, dem Oryzanin fehlt, kann das Leben des Tieres nicht längere Zeit erhalten.

4. Die künstlichen Futtergemische aus Eiweiss, Kohlenhydrat, Fett und Salzen, ohne Oryzanin, konnten das Leben des Tieres nicht längere Zeit erhalten.

5. Hunde konnten nicht mit ausgekochtem Fleisch und geschältem Reis existieren, und nach 3 bis 4 Wochen waren sie vollständig abgemagert. Wenn man aber so abgemagerten Hunden täglich 3 g alkoholischen Extrakt oder 0,3 g Oryzanin zuführt, so werden sie bald geheilt.

6. Die Verbreitung des Oryzanins in verschiedenen Nahrungsmitteln ist ziemlich gross. Da aber der geschälte Reis bei uns in Japan ein Hauptnahrungsmittel des Volkes bildet, so kann der Mangel an Oryzanin

sehr oft eintreten, besonders bei denjenigen Leuten, die immer von bestimmten, wenigen Nahrungsmitteln leben und keine Abwechslung haben, wie die Leute in Werkstätten, Läden, Gefängnissen usw.

Was wird nun der Effekt des Mangels an Oryzanin bei Menschen sein? Viele Mediziner behaupten, dass die Beriberikrankheit durch geschälten Re'is verursacht wird<sup>1</sup>. In den Philippinen ist man sogar so weitgekommen, dass das Essen des geschälten Reises verboten wird.

Wir wollen das Studium der Beriberi, besonders der Beziehungen zwischen dieser Krankheit und Reismahrung, Medizinern überlassen und beabsichtigen, später über die chemische Natur des Oryzanins und seine physiologische Funktion bei Tieren weitere Aufklärung zu geben.

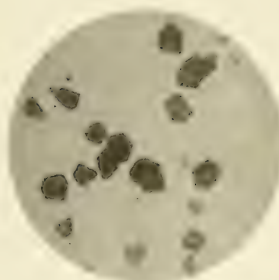
1. Vergleiche die Arbeiten von BREAUDAT und DENIER: The use of rice bran in the prevention and cure of beriberi. *Annales de l'Inst. Pasteur* 25, Nr. 2, S. 167 bis 189, 1911.—BREAUDAT, Studies on the protective power of bran in a polished rice diet. *Bull. soc. Path. Exot.* 4, Nr. 7, S. 493 bis 502, 1911.—W. P. CHAMBERLAIN, H. P. BLOOMBERGH and H. P. KILBOURNE, Study of the influence of rice diet and of inanition on the production of multiple neuritis of fowls and the bearing thereof on the etiology of beriberi. *Philippine Journ. Sci. B. Med. Sci.* 6, Nr. 3, S. 177 bis 209, 1911.—G. HEISER, Practical experience with beriberi and unpolished rice in the Philippines. *Philippine Journ. Sc. B. Med. Sci.* 6, Nr. 3, S. 229 bis 233, 1911.—H. D. W. GREIG, Rice in relation to beriberi, in epidemic dropsy in Calcutta. *Sci. Mem. Med. and Sanit. Dept. India n. ser.* 1911, Nr. 45.—C. TOYAMA, Rice and Beriberi. *Zeitschr. f. med. Mikroskopie* Nr. 104, Dez. 1911.



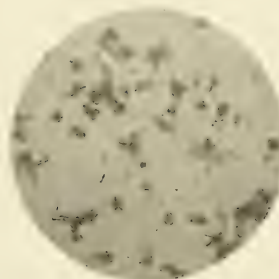
477



I  
Oryzanin pikrat  
(aus Wasser.)



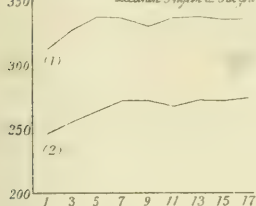
II  
α-Säure  
aus Roh-Oryzanin (I).



III  
β-Säure  
aus Roh-Oryzanin (I).



## Versuche mit Tauben.

Versuch II. Geschältes Reis mit Roh-Cryzanin (i)  
Lecithin, Phytin u. Fatzen

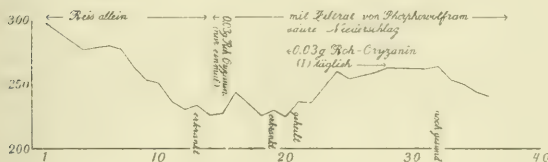
Versuch III.

Geschältes Reis mit Lecithin,  
Phytin u. Fatzen, ohne  
Cryzanin.

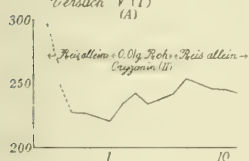
Cryzanin. (I) per os.



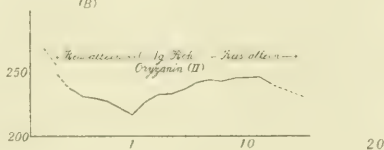
Versuch IV



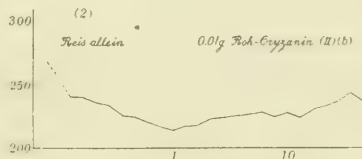
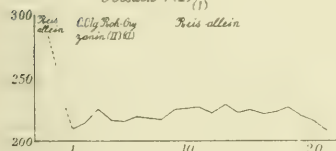
Versuch V (I)



(B)



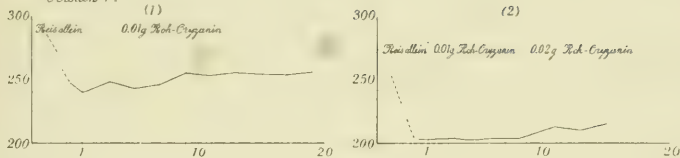
Versuch V (II) (I)



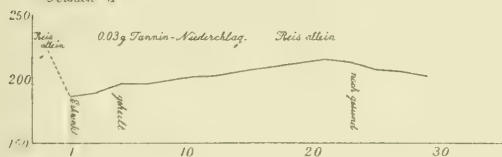


Versuche mit Tauben

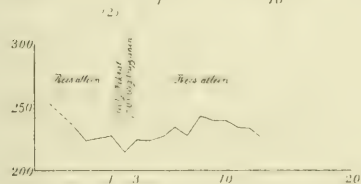
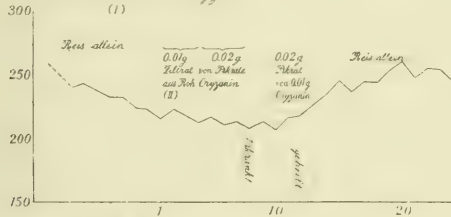
Versuch V.



Versuch VI



Versuch VII Reines Cyanarin





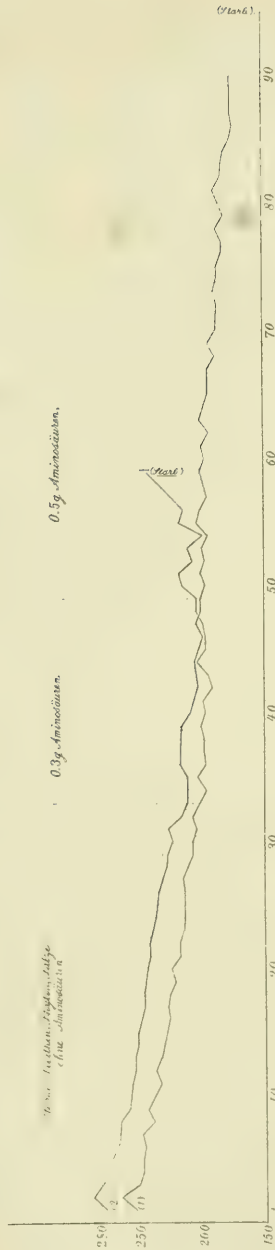
Verband mit Tannin

Versuch V. Spaltungsprodukte des Eucisäure

aus, Indan-Naphthalin, Indol  
eine Substanz

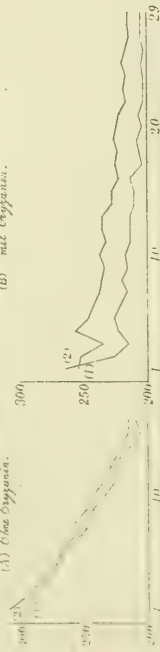
0.3g. Aminosäuren.

0.5g. Aminosäuren.

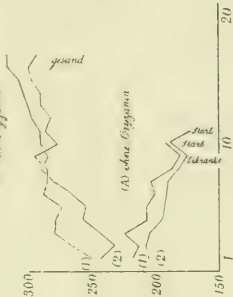


Versuch K.

Indan, Naphthalin, Indol, Indol  
(A) ohne Organische, (B) mit Organischen.



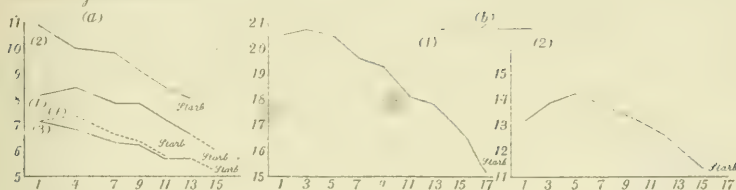
Versuch VIII  
Indan, Naphthalin, Indol, Indol  
(A) ohne Organische, (B) mit Organischen.



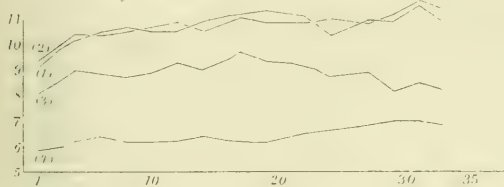




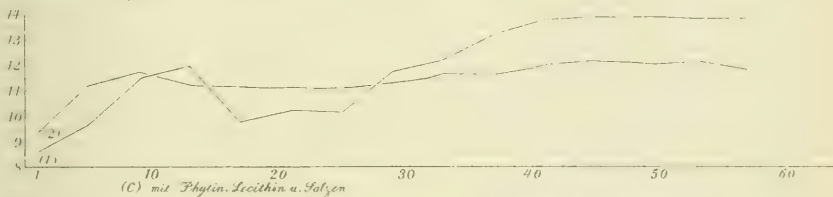
Versuch I. Geschüttelter Reis



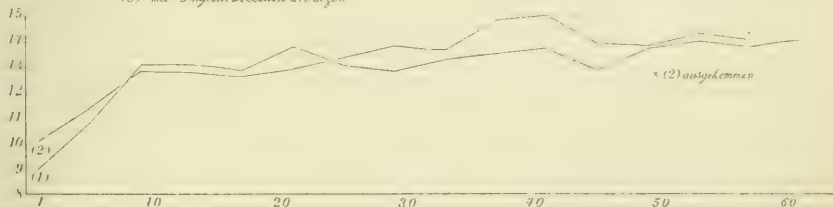
Versuch II. Ungeschüttelter Reis



Versuch III. Geschüttelter Reis mit atchulestem Extract der Reiss



(c) mit Phytin, Lecithin u. Salzen

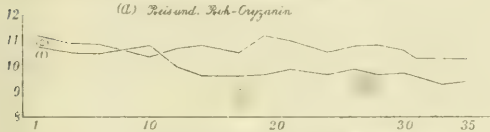




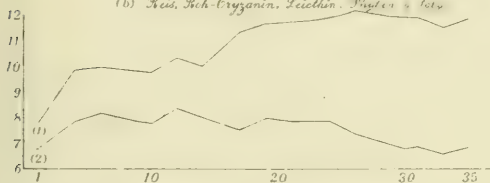
Versuche mit Mäusen.

Versuch IV. Geschälter Reis mit Roh-Cryzarin (1).

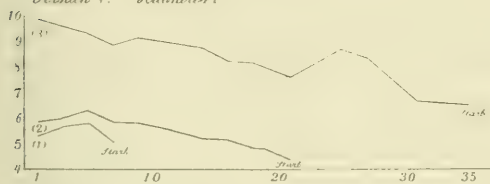
(1) Reis und Roh-Cryzarin



(b) Reis, Roh-Cryzarin, Lecithin, Biotin & Fett

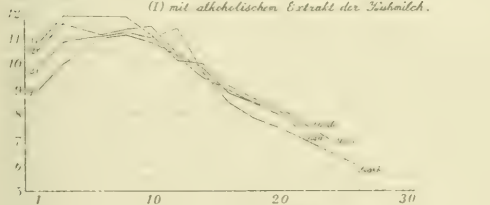


Versuch V. Hühnerier



Versuch VI. Kuhmilch.

(1) mit alkoholischen Extrakt der Kuhmilch.

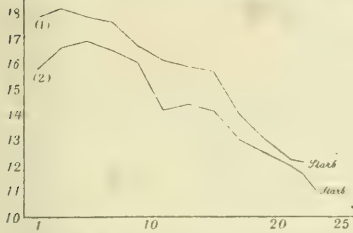


(2) mit getrockneter Milch

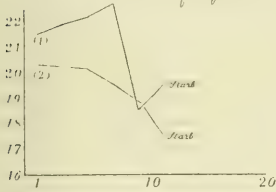




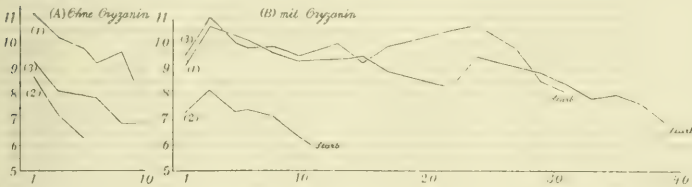
Versuch VII. Geschälter Reis mit Margarin.

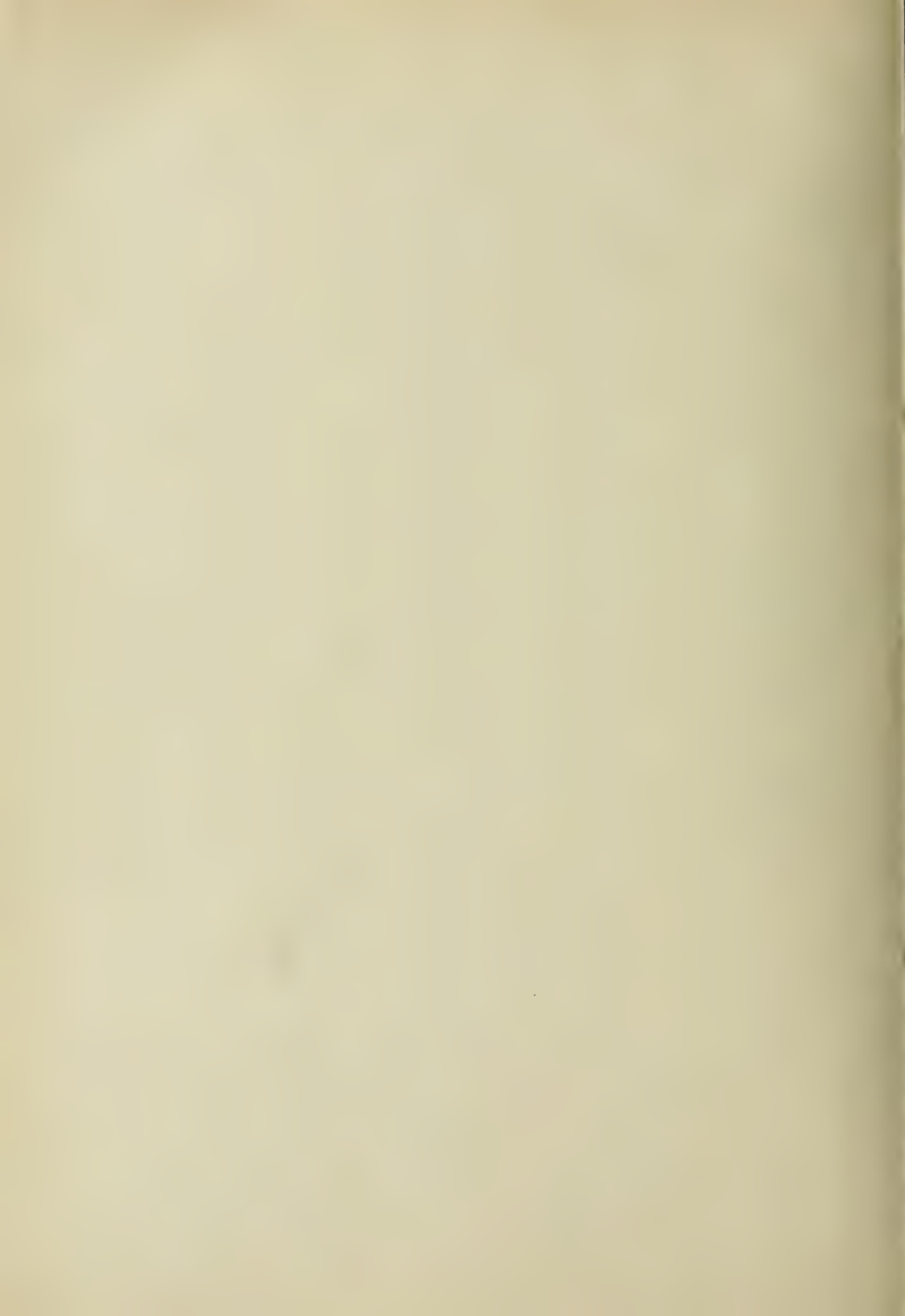


Versuch VIII. Geschälter Reis mit alcoholischem Extrakt von Pferdefleisch.



Versuch IX. Stärke, Kasein, Lecithin und Salz.





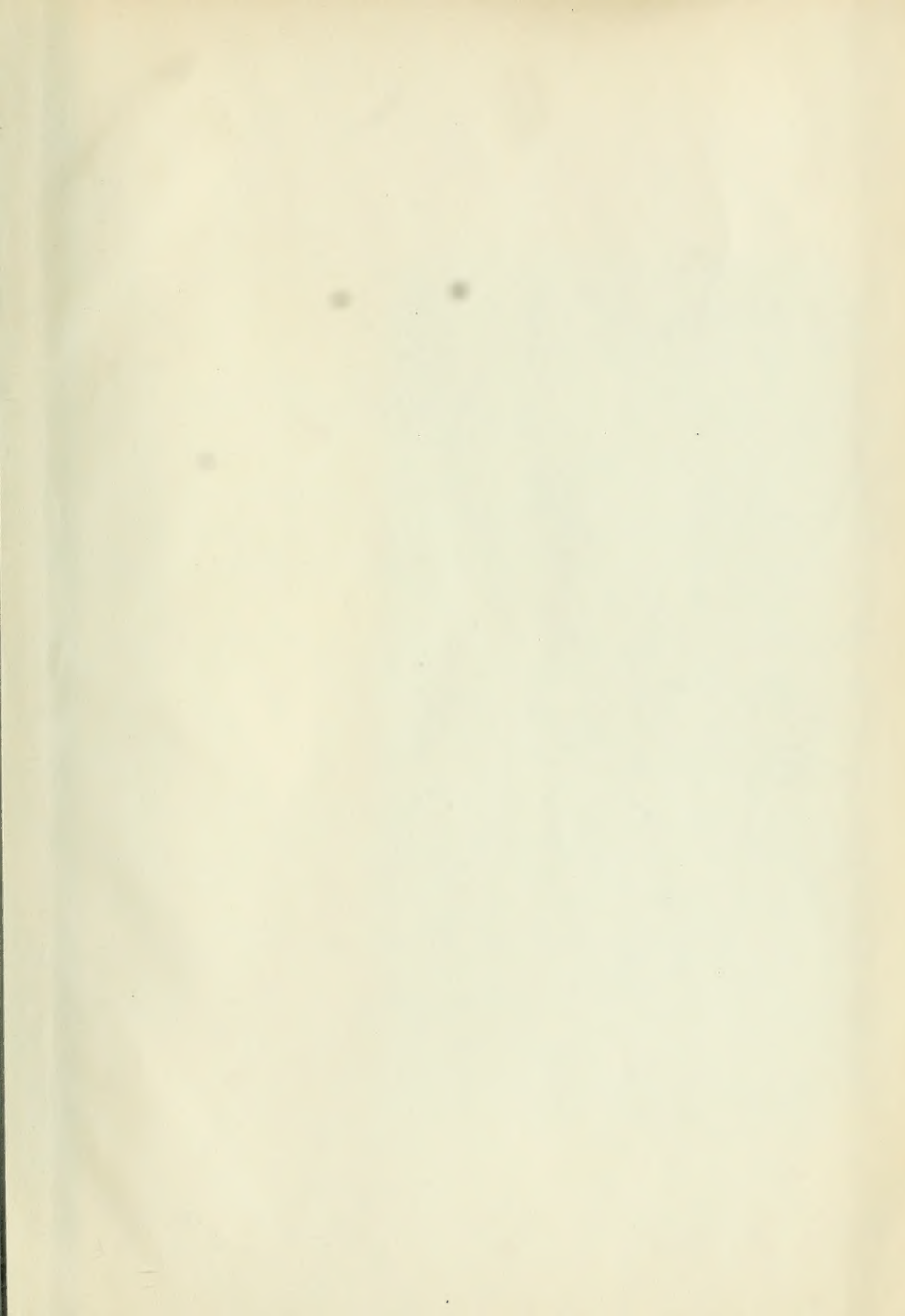


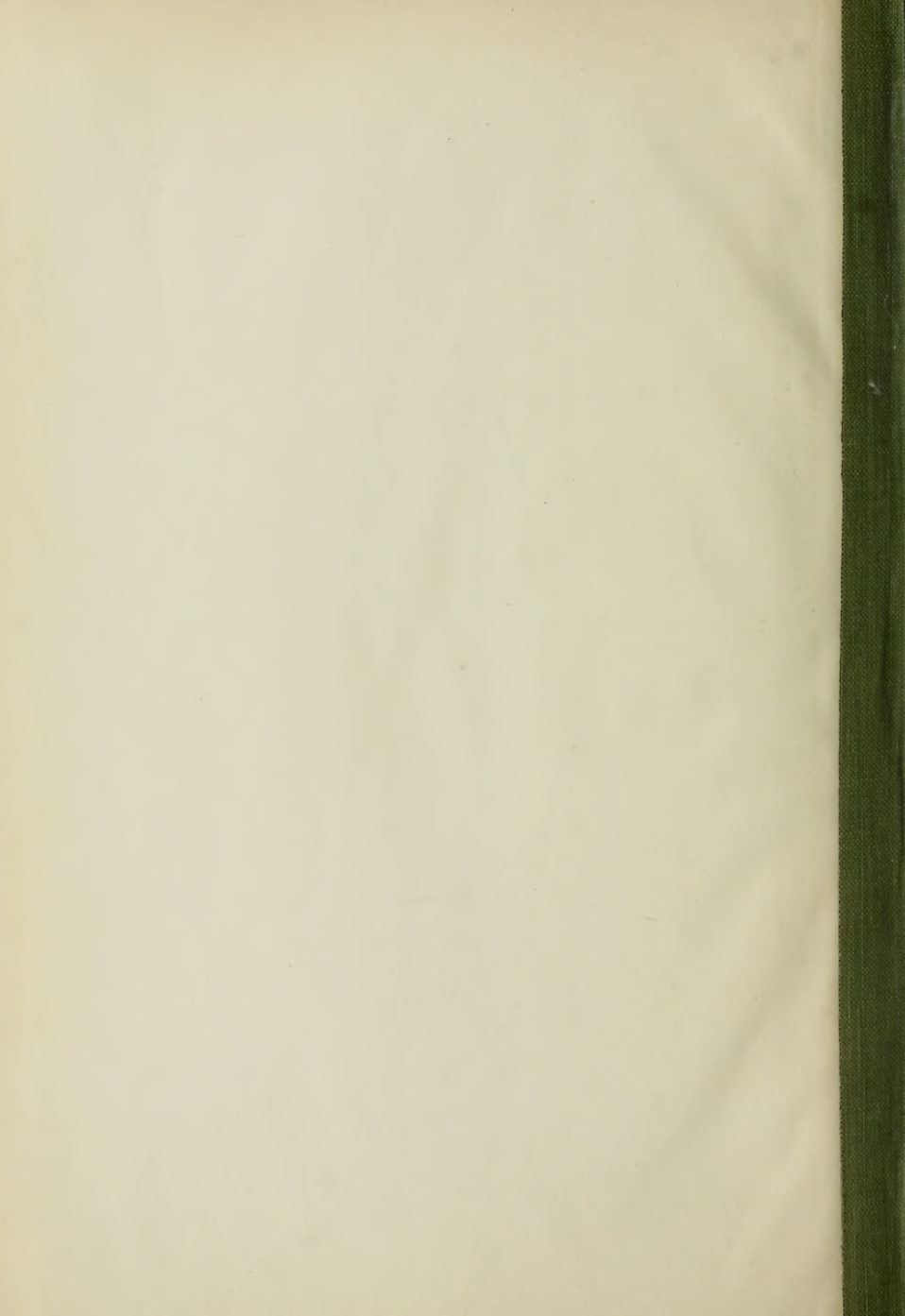
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